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Experimental Atheromatosis

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of Medicine by James Z. Davis

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### Experimental Atheromatosis

The production of lesions in the aorta of experimental animals pathologically similar to those occurring spontaneously in the aorta of man has created renewed interest in this morbid anatomy because of two reasons. It has offered some clue to the etiology of this condition about which little is known and it has offered optimum opportunity to study the pathogenesis of this lesion. Scientific control of any morbid process will only be arrived at by a clearer understanding of all of the etiological factors and the mechanism by which they operate. The goal of all workers has been to clarify these two factors. The extent to which that has succeeded will be reviewed.

From the time when the earliest records are available on arteriosclerosis, shortly after the time of Vesalius until Ignatowski (1908) noted yellow intimal thickening in some rabbits which he had been feeding a high protein diet will be covered briefly. The ancients were probably familiar with arteriosclerosis but none of them have left any records to show that they were interested to any extent in this lesion. Aristotle speaks of "bone in the heart" which gave rise to considerable speculation. (9) That this lesion did exist in ancient people is verified by the work of Marc Armand Ruffer who, working with the Cairo Medical School, examined many mummies and found this lesion to be possibly more frequent at that time than it is at the present. (35) From the time of Vesalius up until about the middle of the eighteenth century there was little written about arteriosclerosis. What was written about the arterial lesions was largely merely a passing reference about an observation. Most men who left any records during this time were familiar with

"ossification" of the arteries but a great deal more time was consumed in studying aortic aneurysms. The only description of an arterial lesion worthy of note made during the seventeenth century was made by Brenner. Describing an arteriosclerotic aorta Brenner said, "the internal coat in several places was ruptured, lacerated and rotten like fruit, and hurt the fingers when thrust in it, from the roughness of the bone". Johann Friedrich Crell (1740) was the first man to outline the pathogenesis of atherosclerosis. He was also the first to note that the calcifications of the arteries were not bone but were from pus. His ideas were that sluggish blood tended to obstruct the arteries and this led to suppuration of the wall of the artery, this accumulation of pus tended to separate the inner and the outer coat, the liquid fraction was absorbed, the particulate matter formed scales. He was also one of the first to suggest etiology. He maintained that these changes occurred in the aged and the intemperate young.

Albrecht von Haller (1755) was one of the first men to describe the atheromatous character of arterial lesions. From some of his necropsy experience he described some soft lesions which elevated the aortic intima and which when cut into yielded a yellow fluid. He found other like lesions which were harder and had no liquid in them. These changes he believed to be a part of the process of arteriosclerosis.

Morgagni (1761) described in minute detail the changes found in the arteries. His description dealt not only with the aorta but he described calcareous deposits in the subclavian, carotid and iliac arteries. He speculated on how these changes might hinder the passage of blood through the vessels but he drew no relation between the lesions and

arteriosclerotic gangrene. He was also one of the first to note that arteriosclerosis was not an inevitable senile change, he describing a necropsy on a lady ninety one years old who did not show any arteriosclerosis lesions. He speculated at length upon whether or not the calcareous particles were bone or not, finally inclining to the latter. Morgagni was the first man to point out the relationship between what is known today as an atheromatous abcess and a dissecting aneurysm. Scarpa (1804) a student of Morgagni's, was the first man to reason that a degenerative lesion of the aortic wall preceeded an aneurysm.

Brihat (1801) came to the conclusion that the "ossification" in the arteriosclerosis was in the inner layer. He says nothing about atheromatous changes except in connection with aneurysms. He definitely connected arteriosclerosis with senility by observing that arteriosclerotic lesions occurred in seven bodies out of every ten in the aged. Laennec (1819) wrote at length on aneurysms. He believed that the lesion in arteriosclerosis lay between the intima and the media.

Edward Jenner, Caleb Parry and Matthew Baillie correlated coronary arteriosclerotic changes with the disease angina pectoris. None of these men contributed anything to the pathogenesis of arteriosclerosis. Hodgson (1815) considered atheromatous lesions true pus, he believed the lesion to occur in the inner coat. Hodgson wondered about the chemical composition of the calcareous material occurring in the aorta and was the first man to have a chemical analysis made of them. He had a chemist friend analyze these lesions and found them to be 65.5% phosphate of lime and 34.5% animal matter. No carbonate of lime was

found so Hodgson considered the material to be earthy and not true bone. He was the first man to note what is today called Monckeberg sclerosis. Hodgson was puzzled as to whether or not these changes in the arteries were merely a consequence of life, that is were an inevitable occurrence in the aged or whether they were a distinct disease.

Carswell and Hope (1837) were alive to the pathology brought about by arteriosclerosis publishing articles on gangrene of the extremities as a result of "osserus" change in the arteries. They were the first to note the relationship between encephalomalacia and arteriosclerosis of the cerebral vessels. They did not however contribute anything to the pathogenesis of the arterial lesions.

Lobstein of Strasbourg (1829-33) is to be remembered as being the one who first coined the word arteriosclerosis. He had Lassaigne make an analysis of calcified arterial plaques in which he reported mucus animal matter 50%, carbonate of lime 2% and sulphate of lime in traces.

Jean Cruveilhier believed that the pultaceous matter in the atheromatous lesions were carried by the blood. He believed that calcification was a protective reaction to atheromatous arterial change. He was one of the first men to compare arteriosclerosis with the physiological closure of the umbilical vessels, calling attention to the fact that the same thing occurs when an organ atrophies, but he could not decide if it was the atrophy which caused the arterial obliteration or whether it was the arterial obliteration which caused the atrophy.

Carl Rokitansky in Vienna in the 1840's contended that blood dyscrasias were one of the etiological factors in atherosclerosis.

This dyscrasia terminated in two forms, atheroma and ossification.

He thought that this substance was deposited on the intima rather than in it. He said, "the deposit can not be regarded as an exudate from inflammation of the artery. It is an endogenous product from the blood mass, and one derived preponderantly from the fibrin of the arterial blood". He did not attempt to name the blood condition responsible for arteriosclerosis. He thought that it was not due to old age alone but that old age predisposed to this lesion.

Virchow (1856) was one of the first histopathologists to contradict Rokitansky when he maintained that the lesion occurred in and not on the surface of the blood vessel. He described the swelling of the ground substance of the connective tissue of the intima and an increase in the cellularity in these lesions, these changes occurring before the fatty metamorphosis began. He observed that this lesion occurred more often in the straight and elastic arteries close to the heart where tearing and stretching is more prone to take place. He favored the opinion that a blood dyscrasia must also be present to carry out the complete pathogenesis. (9) It is interesting, that later on Aschoff in 1924 interpreting the work of Virchow said that Virchow considered all of these changes as being a defensive reaction on the part of the artery to some noxious influence. (2) Rindfleisch a pupil of Virchow's elaborated his ideas. In this work the original lesion was an overgrowth of connective tissue of an inflammatory etiology which in turn was due to the irritation of the blood pressure on the affected point of the intima. This scarring of the intima resulted in poorer nourishment to the intima underlying the thickened spot which resulted in fatty degeneration of the

connective tissue cells, and impregnation of the intercellular substance with calcium salts. The etiology was suggested as being free living, advanced age and alcoholism. (9)

The work of George Johnson as English histopathologist is of interest in that he contributed some ideas on etiology. He had observed the hypertrophy of the arterioles in the contracted kidney in Brights disease. He thought that thickening of the arteries was due to poor renal function which allowed the metabolites to pile up in the blood resulting in more work for the heart and a simultaneous hypertrophy of both the heart and the arteries. Gull and Sutton at about the same time contradicted Johnson in that they did not believe that it was a hypertrophy of the muscular coat of the arteries which caused this vascular disease but that it was a hyaline fibroid change in the muscularis that resulted in a narrowed lumen.

Although luetic endarteritis had been described a number of times by many authors beginning with Raymond Vieussens of Montpellier in 1715 it remained for O. Huebner in Germany in 1874 and F. H. Welch in England in 1875 to clearly distinguish between luetic aortitis and arteriosclerosis. Schmorl (1907) of Dresden was the first man to discover the *Treponema pallida* in the lesions of aortic mesaortitis. (9)

Klotz in a paper published in 1906 goes over some of the earlier experimental work and concludes that there is only one type of arteriosclerosis that can be produced in laboratory animals similar to any lesion that occurs in man and that is the one produced by the intravenous injection of adrenalin chloride. He goes over the earlier work of Gilbert and Lion (1889 and 1904) in which they had apparently



produced intimal lesions by the inoculation of bacteria and their toxins. In commenting on this he is of the opinion that their results were not consistent enough to warrant any hope of solution of the problem along these lines. (17) Later on the same year he published an account of some of his work on the production of arterial lesions in laboratory animals. In this paper he disagrees with Thoma who believed that all true arteriosclerosis began in the intima and that the Monkeberg type of lesion should not be called arteriosclerosis. Klotz was of the opinion that this the Monkeberg type which could be produced in laboratory animals by the injection of adrenalin chloride was as much an arteriosclerosis as was that of intimal origin.

Klotz reviews the work of Malkoff and Fabres who produced lesions by pinching the arteries either through the skin or opening the skin and injuring the vessel directly. Malkoff ligated the carotid artery in two places then placed increased pressure on it then allowed the blood to return to it. Malkoff claimed that this procedure produced arterial lesions but Czyhlarz and Hebling repeated the experiment and reported that the arterial change was simply an inflammatory one extending inward from without. (18)

Kurt, Ziegler and Klotz were the first to compare the lesions induced by adrenalin and to point out their resemblance to the Monkeberg type of arteriosclerosis. The degeneration of both instances is of the nature of fatty emtamorphosis of the involved tissue, which later on undergoes calcification. Ziegler held that such lesions are of a nutritional character or rather due to lack of nutrition. Torri and others on the other hand regarded them as the outcome of heightened blood pressure

while Fischer considers the process as pure necrosis due to the direct action of the drug on the muscle cells. Observations in favor of the degenerative or necrotic theory have been recorded by Braun. (18)

Klotz used adrenalin chloride, alone, adrenalin chloride with nitroglycerine, barium chloride, saline suspensions of *Bacillus typhosus*, *staphylococcus*, and diphtheria toxin. He used adrenalin chloride 0.3 cubic centimeters to 2.0 cubic centimeters of a one to one thousand solution. He found that with this treatment many of the animals died with acute pulmonary edema during the experiment and that the best results were obtained by using large doses at intervals of two to three days. This type of treatment caused diffuse sclerosis of the media of the elastic, musculo-elastic and muscular arteries rather consistently in two to three weeks. Aneurysmal dilatation of the thoracic aorta was frequently observed in these animals. The results obtained with the barium chloride were exactly the same as those produced by the adrenalin chloride. The similarity of the two lesions is such that they can not be distinguished between. When adrenalin was used in conjunction with nitroglycerine which pharmacologically should contract the vasoconstrictor effect of the adrenalin the same lesion occurred in the media, however it was not near so severe. The inoculation of digitalin intravenously produced a lesion which resembled the mild adrenalin lesions. In regard to the preceding experiments Klotz says, "In no instance have I found a primary change occurring in the intima after any of the above treatments, though in one of two specimens I did note a slight thickening of the intima at the margin of the aneurysm.

*Bacillus typhosus* and *Streptococci* was used to induce lesions

by their intravenous inoculation into rabbits. With this procedure the first part of the pulmonary artery and the ascending limb of the aorta showed warty thickenings of the intima. There were no aneurysmal sacs nor any sign of calcareous degeneration of the media. Microscopically there was a fatty degeneration of the subendothelial tissue while there was however much connective tissue advancing into the degenerated area. A small celled infiltration was wanting as was also any sign of calcification. At the areas of thickened intima it was found that the internal elastic lamina had split into several parallel layers and the fibers so formed were stretched between the proliferating cells.

The inoculation of diphtheria toxin caused no proliferative changes but simply a degenerative change in the media of the arteries not unlike the early changes in those animals given inoculations of adrenalin. (18)

Ignatowski in 1908 published the results of his experiments on the effect of animal food on the rabbit. By feeding milk and egg yolk he produced various lesions of which atheroma of the aorta, cirrhosis of the liver and enlargement of the adrenals were the most conspicuous. In the following year Starakadomsky and Ssobolew by using the same diet and animals produced the same results. They also reported atheromatous lesions in the inostrate, the carotids the subclavians and the iliac arteries. Ignatowski was of the opinion that it was the protein in the diet which produced these results. Stukey confirmed Ignatowski's results and attempted to determine exactly what it was in the diet which produced these lesions. He found that these lesions could be produced only with egg yolk. In following up

experiments he fed rabbits various animal and plant fats, fish liver oil, beef fat, and sunflower seed oils with negative results. He was successful in producing atheromatous lesions of the aorta in rabbits by feeding them on brain. Wesselkin fed rabbits on various diets and concluded that it was the cholesterol in the diet which produced the atheromatous change. Anitschkow and Chalатов were able to produce atheromatous lesions in the rabbit aorta by feeding of cholesterol.(3)

Adler, who was one of the first man in this country to conduct experiments on atheromatosis began his experiments in 1908 and published his first reports in 1914. He used dogs for the most part of his experiments. The first part of his work dealt with the introduction by hyperdermic inoculation of noxious substances either subcutaneously or intravenously. These substances were triethylacetate of lead, nicotine, diphtheria toxin, live cultures of *Staphylococcus aureus* in saline solution, live cultures of *Streptococcus pyogenes albus*, *Bacillus coli*, *Baccillus typhosus* and sodium sale of chondroitin sulphuric acid. He used all of these on dogs all with negative results so far as producing any aortic lesion was concerned. With the feeding of cottonseed oil to dogs he was able to produce fine fatty striae on the aortic wall in two dogs out of three. It is interesting here that the cholesterol content of the blood was determined and found to be 0.00228 which I presume means 228 Mgm. percent and is markedly above the normal as compared with other figures the author gives. Uranium nitrate administered subcutaneously was combined with oil feedings with negative results. Two dogs were fed 0.5 of a gram of pure cholesterol every other day or two and a half and one and half months respectively. In both instances the

aorta of these animals showed fine fatty striae in the intima. He was apparently able to produce these intimal lesions in dogs by the feeding of dilute hydrochloric acid. At this time the stress and strain of an active life were supposed to be an etiological factor in arterial pathology so Adler placed some of his animals on a treadmill giving one animal adrenalin and using the other as a control. So far as aortic pathology was concerned his results were negative. He did however observe that the dog which received the adrenalin was tireless and did not develop glycosuria which the control animal did develop.

Concerning the histo-pathology of the lesions in this series Adler reports that the very earliest lesion is a proliferation of rather large flat cells in all probability of endothelial origin. No cells resembling macrophages or amoeboid leucocytes could be found. Simultaneously with the proliferation of these cells elastic fibers begin to be split off of the elastica interna between these fibers there develops a more or less swollen succulent connective tissue. At the base of the lesion these endothelial like cells line up with their long axis perpendicular to the elastica interna on which they lie. The fibers of the media immediately adjacent to the lesion begin to give way and this area becomes swollen. No aggregation of leucocytes or anything resembling an inflammatory reaction could be made out. With the first appearance of the proliferating cells Sudanophil droplets begin to appear in the upper portions of the proliferating cells and also lying free both above and between them. Very soon these same droplets appear in the media and the adventitia. As the process proceeds the droplets become larger but never of the size as described in man or the rabbit. In conclusion Adler

advances no theories or ideas as to the mechanism involved in the production of these lesions. He observed that the Sudanophilic droplets found in the lesions are optically inactive and therefore are probably not cholesterol. (1)

Bailey who fed rabbits cholesterol and egg yolks in different amounts noted an enormous intimal thickening. In the earliest lesions the greater part of the double refracting fat was intracellular. A collection of fine fat droplets is however seen along the intimal layer of elastic tissue this fat being for the most part extracellular. The fat containing cells especially in the early lesions vary considerably in size, form and fat content and it seems possibly to follow gradations from the spindle cells with a few fat droplets to the large cells filled with fat. It is believed that the fibroblasts in the intima as elsewhere play a part in taking up the fat, but from the appearance of gradations mentioned it does not necessarily follow that the fibroblasts swell up to form the large cells described. As the lesion progresses there is an increased proliferation of fibroblasts and an abundant formation of collagen and fine elastic fibers especially in the upper portion of the lesion. The large collection of fat containing cells in the lower portion breaks down and areas of degeneration are formed in the lower intima with an abundant deposit of cholesterol crystals and in some cases calcification capped by fibrous tissue. The lesion is mainly confined to the intima but not entirely so. Often both intracellular and extracellular fat is seen extending outward half way through the media. The same degenerative steps may follow this early change in media as it did in the intima.

Bailey summarized his work as follows: "The feeding of egg yolk

or pure cholestrol to rabbits produces an abundant deposit of anisotropic fat in the various organs. From this deposit characteristic lesions secondarily result in certain organs. Prominent amount these lesions is an atheroma of the aorta very similar to the gross and histologically to the human lesion. Lesions of other vessels are also produced conspicuous amount which are those of branches of the pulmonary artery. There is a large deposit of anisotropic fat in the liver which produces a cirrhosis. Enlargement of the adrenals occurs probably due to the storage of fat. In a certain proportion of rabbits conspicuous lesions are produced in the kidneys consisting of nodular deposits of anisotropic fat in the medullary portion, the fat being contained for the most part in endothelial cells and fibroblasts in the interstitial tissue. Later softening occurs in these areas as in the aorta; the cells break down; there is an abundant deposit of cholesterol crystals, some calcification and a proliferation of connective tissue. Scars frequently extend outward from these lesions through the cortex but the nodular deposits of anisotropic fat are never seen in the cortex. It is impossible to determine definitely from these experiements whether or not these deposits are dependent on preexisting interstitial lesions". (3)

Klotz in a review of the material up to 1914 was of the opinion that experimental atheromatosis was yielding disappointing results. His chief criticism of what had been reported so far was the lack of consistent results. He returned to postmortem material and wrote a paper on the necropsy study of arterial lesions in twenty three bodies in age from ten to fifty six in all of which death was caused by an acute infectious disease. He found in this study that the reactions of the intima in many

acute infectious diseases may occur independently though simultaneously with the inflammatory reaction about the vasa vasorum. The intimal reactions of the aorta are more marked about the mouths of the smaller vessels, particularly the intercostals but they do not usually advance along the lumen of the branching vessel. The reaction in the intima is an inflammatory one, in which the wandering cells, lymphocytes, some plasma cells and polymorphonuclear leucocytes may be best observed when the intima is cut flat. This inflammatory process is accompanied by progressive as well as by degenerative changes in the tissues of the intima. Repair is accomplished by a proliferation of the connective tissues of the inner layer of the intima and may show some hyperplasia of musculo-elastic layer. Splitting of the internal elastic lamina into multiply bands occurs in reactions of a purely inflammatory nature in the absence of hyperplasia of the deep muscle layer. Fatty degeneration is a common accompaniment of the pure connective tissue thickening of the intima, and is found to involve particularly the connective tissue cells, the elastic fibers and the endothelial-like cells found in the intima, deep areas may also show medial involvement. The late stages of the lesion can't be differentiated from the process of atheroma with superficial endarteritic thickening. It is superfluous to differentiate atheromatous softening of the intima by the particular tissue cells which show fatty degeneration and which on subsequent destruction liberate fatty materials in the intima of the arteries. The etiology of these changes is probably bacterial toxins, once these have set in nutritional changes may occur which are important. "The entire process may be classed as one of arteriosclerosis in which proliferative and degenerative reactions are closely associated." (19)



Again in a paper published in 1915 Oskar Klotz called attention to the fact that the early degenerative changes in atherosclerosis was an important factor that was being generally overlooked. After describing this hyaline change in the ground substance of the intima together with the splitting of the fibers of the internal elastic lamina he summarizes by saying. "Thus in truth whether the important factor in arteriosclerosis is of a mechanical, bacterial or chemical character, the degenerative reactions in each plan an important part in the progress of the disease. In the later atheromatous softening with its complex chemical constituents, the deposition of calcareous salts is directly associated with the presence of these fatty bodies. Such atheromatous areas have their beginning in and their progressive enlargement due to the destruction of a variety of cells which by disintegration liberate their fat content." (20)

Newburgh and Squires (1923) thought the term atherosclerosis should be clarified before they reported their work, because of its clarity it is here given verbatim. "The term atherosclerosis as is used in dealing with the diseases of arteries occurring in human beings is intended to designate a primary lesion of the intima and to exclude lesions of the intima which result from disease of the media and vasa vasorum. The process presents itself to the naked eye in its most characteristic stage in the form of discrete or confluent slightly raised pale yellow opaque plaques and streaks. This earlier form of the lesion may later be altered by softening of the contents of the nodule and the loss by ulceration of the caseous material thus formed. The deposition of calcium salts in large amounts converts the base of

the ulcer into a hard but brittle depression. But these well recognized gross appearances are not always sufficient to permit the observer to distinguish between primary atherosclerosis and other vascular lesions which affect the intima secondarily, in particular syphilis of the aorta; and in experimental work, the microscopic features should be used in reaching a decision. The histological picture presented by this primary disease of the intima depends on the stage at which it is seen.

The intitial lesion consists essentially of a necrosis or fatty degenerative change in the endothelium or the fibroreticular cells of the intima. The yellow elastic fibers are separated and pushed apart by the swollen fatty cells. With increasing injury the elastic fibers undergo a granular degeneration and break up into a granular detritus.

Following the stage of primary injury, a regenerative compensatory fibrosus occurs with the production of new elastic fibers. The growth of new elastic fibers is often marked and usually occurs chiefly on the lumen side of the lesion so that the degenerated area comes to appear to be located nearer to the media than to the lument.

A secondary degeneration of this sclerotic area occurs and may result in rupture of the atheromatous patch into the lument of the vessel forming the so-called atheromatous ulcer. In advancing lesions there is a progressive fatty degenerative change involving not only the intima but extending into the fibrorecticulum of the media. At any time calcification may be an added factor. The two processes, the degenerative and the regenerative-compensatory go hand in hand and in old progressive cases may lead to the most marked changes of the vessel wall extending even to the adventitia.

When one wishes to determine whether an experimental vascular lesion is atherosclerotic in type it is always desirable to examine the earlier stages of the lesion. In primary disease of the intima it will be noted that this coat has undergone great thickening and that its increased width is chiefly due to the presence in it of large cells loaded with fat imbedded in a hyperplastic elastic tissue. The coexistence of enormous amounts of fat and the hyperplasia of the elastic tissue stamp the lesion in question as a true disease of the intima, an atherosclerosis".

In summarizing the work that had been done before them Newburgh and Squires came to the conclusion that of all the methods tried only the intravenous injections of bacteria and the feeding of animal food has consistently resulted in the production of experimental atherosclerosis. Animal diets are abnormal for rabbits in at least two ways. They contain an excess over herbivorous diets of both protein and cholesterol. A number of workers have tried to prove that the vascular lesions occurring in rabbits fed animal diets were caused by the cholesterol. It has however not yet been shown that a simple hypercholesterolemia can produce atherosclerosis. The literature contains no record to date (1923) of a systematic investigation of the possible effect of the protein in the diet on the arteries of rabbits. Consequently they undertook the following experiments.

Two different diets were made up thusly. The first contained one thousand grams of powdered beef from which the lipid had been removed mechanically; two thousand grams of flour bran mixture; twenty grams of sodium chloride; fifty grams of baking powder and then spread thickly and baked. The second diet was the same except only half the

amount of beef was used as in the first diet. On analysis the first diet contained 36.2% protein, the second 26.8%. In addition to the above diets the animals were allowed one hundred grams each of fresh greens once a week.

Twenty four animals lived for four weeks or more on the diet containing 36% protein. They may conveniently be divided into three groups by the duration of the experiment. In the first group are ten animals that lived from four to eight weeks on the diet. Two of these rabbits showed early intimal disease. The remaining eight rabbits ate the high protein mixture from eighteen to thirty six weeks and all of them presented marked and extensive atherosclerosis.

Fifty one animals were fed the diet containing 27% protein. These rabbits fall naturally into two groups, forty that lived for less than six months on the diet containing 27% protein showed atherosclerosis of the aorta; whereas eight of the eleven animals that ate this diet for more than six months presented aortic lesions which were grossly and histologically typical of true atherosclerosis. The lesions in seven of the eight rabbits were advanced and severe. The description of the lesions corresponds closely to the criteria laid down by the authors previously.

In the discussion of the spontaneous atherosclerosis these authors present figures from several sources such as Rosenow finding atheromatous lesions in three rabbits out of a total of 1,548 animals examined; Loeb examined 483 finding no arterial change. Out of the 116 rabbits in this study none showed spontaneous intimal changes.

In regard to the age of the animals being a factor it was

found that the control rabbits which showed no arterial change were in general older than the experimental group.

Infection, that is spontaneous infection is not a factor according to these authors because they report finding many of the common infections among the controls during the experiment. There was no effort made to isolate an animal when it became sick.

In discussing the role of cholesterol the authors calculate the amount of cholesterol ingested by their animals on this diet would be a maximum of 28Mgm. daily. They find that the amount of cholesterol consumed by their rabbits as being 22% of the amount fed by Starokadomsky 44% of that used by Wesselkin and 58% of that eaten by Saltykow's rabbits. These amounts which were fed by Newburgh and Clarkson were then much less than the minimal amounts said by previous workers to be necessary to produce atherosclerosis.

In summarizing their experiments they say. "our experiments have shown that the prolonged ingestion of excessive amounts of protein by rabbits will result in extensive atherosclerosis of the aorta and several other arteries. Study of sources of error has demonstrated that the atherosclerosis found in the rabbits that ate the high protein diets was not spontaneous; was not the effect of laboratory environment, age or infection; could easily be distinguished from the spontaneous calcification of the media; and was not caused by the small amount of cholesterol in the diet". (28)

Aschoff in his lecture in this country in 1924 gives quite comprehensively his ideas on the pathogenesis of human atherosclerosis. Aschoff goes back to what he calls the concept of Virchow which is, the first morbid change consists of a swelling of

the ground substance of the intimal connective tissue. This swelling must be due to an imbibition from the blood stream of the aorta. It is recognized microscopically by the increased width and homogeneity of the connective tissue spaces. Aschoff believed that Virchow has been misunderstood, he says, "he Virchow, thought not of defensive endarterities but of a reparative endarteritis which resulted from the primary injury to the intima determined by its mechanical loosening and the infiltration of blood plasma". Following this there is the deposition of cholesterol esters in the injured intima. It is interesting to note here that he makes no mention of cholesterol having any part in the process.

According to Aschoff there are three age groups. The first group of the ascending period from birth to thirty three years of age; the second of the summit extending from thirty three to forty five years, the last or the descending period from forty five years old to death. In discussing the descending period he makes the statement that, "senile ectasis is a disease of old age which no living individual escapes". He believes that too much stress has been laid on size of aorta in the determination of constitutional types and warns that the dilatation of the aorta which begins in the fourth decade and becomes increasingly more marked should always be borne in mind when one speaks of a normal or abnormally wide or narrow aorta. He had examined 685 bodies of war time material and could determine no relationship between the size of the aorta and the constitution.

Senile overstretching can be attributed to a diminution of elasticity of the aorta. When cut the ends of a young aorta will

contract several centimeters which is not true of old aortas. Young aortas can be stretched several centimeters, old aortas can be stretched practically not at all depending on their age. This loss of perfect elasticity, this increase to elastic resistance is due to a change which the elastic undergoes with age, a change which is manifest not only in the aorta but wherever there is elastic tissues, in the bronchi and in the skin for example. Overstretching would probably be much greater were it not that the intima with advancing age and tension becomes invaded with more and more connective tissue. Atheromatosis may be observed at any age but atherosclerosis is observed only during the declining period of life. He observed that senile ectasis may be marked but no sign of atherosclerosis is found. From this it is reasoned that atherosclerosis is an engrafted or very frequently associated or disease of the aorta which is secondary to atheromatosis. With this close association it is necessary to understand atheromatosis before atherosclerosis can be understood. The atheromatosis of infancy and childhood is such an easily reversible process, is so transitory that its pathogenesis is not discussed.

In the atheromatosis of puberty the gross changes are a streaking of the posterior wall of the arcus and the thoracic aorta, these areas may be raised above the surrounding intima or as most usually they are level with the surrounding intima. Microscopically a fatty change occurs deep in the intima consisting of a granular deposition of cholesterol esters in the cement substance of the elastic fibers which comprise the stria terminalis. The entire process can be interpreted only as a sort of loosening and swelling of the cement

substance with a simultaneous deposition of fat droplets. The rest of the intima shows practically no changes or at most a slight loosening and swelling of the tissues. As this process progresses there is an increase in the fat droplets. At the same time there is a diffuse finely granular fatty deposition and precipitation of fat droplets between the elastic and the connective tissue fibrillae of the overlying intimal coat and finally there occurs an accumulation of fat droplets in the intimal cells themselves. In other words the fatty change is both interstitial and parenchymatous, characteristic of which is the predominating interstitial change. With the increase in deposition the whole thing finally reaches the endothelium. " No further changes take place in the atheroma of the ascending period of the vascular system". Retrogression of any of the steps in this process may take place. The cholesterol involved in this process is derived from the blood and it gets into the intima by the simple process of imbibition. Of the physical factors involved it is admitted that the constant tug at the openings of the intercostal arteries must be of some importance because this is a favorite site for atheromatous lesions. "There is a second factor that must be present before these atheromatous spots may appear. This it seems to me, is a sufficient concentration of lipoids especially of cholesterol esters in the plasma. From plasma of low cholesterol content no deposition of lipoids will occur even though mechanical conditions are favorable".

In the atheromatosis of age the site of the lesions is the same in general as it is in the younger groups. The arcus aortus, over the obliterated ductus arteriosus, about the intercostal openings



and about the bifurcation of the abdominal aorta are in the aged as in the young favorite sites for the appearance of intimal thickening. In the older group the color of the gross lesion is different; in the young it is always yellow, in the older group they often are a pale gray color and may be cartilagenous in consistency. Microscopically the changes are the same as they were in the younger group but there is a greater tendency to the overgrowth and hyalinization of connective tissue and in this way the intima is thickened by layer after layer. At first the patches are greyish in color but later assume a cartilagenous white appearance. The process of swelling is accompanied by a more or less widespread fatty change, this begins exactly as in the atheromatosis of puberty in the elastic stria terminalis, it is of finely granular nature and affects in particular the cement substance, gradually an increasing number of connective tissue cells become involved until finally there appears under the more or less swollen and hyalinized surface layer an enormous fatty patch. The mechanism is the same in both cases. "thus we have formulated a conception of atheromatosis common to the ascending and descending period of life. This same process involves two tissues; in one case the young, in the other case the ageing intima which reacts in a totally different manner".

The entire process begins in the elastic stria terminalis, hence there must exist conditions favorable to swelling and precipitation. The conditions are favorable at this point because the elastic stria terminalis is the first densely woven membrane that the invading plasma meets and thereby serves as a filter. In it may be followed the first process of loosening of the fiber net-work of the elastic fibers, it is

not an actual tearing of the fibers but only an apparent one, in that the single fibers are separated more and more from one another as a result of the increasing change in the cement substance.

In concluding Aschoff makes some observations on the part metabolism plays in atherosclerosis. In the aged the cholesterol esters break down forming cholesterol which causes fat necrosis, thus the atheromatous ulcer, when the products of this fat necrosis form soaps particularly with calcium then sclerosis ensues. The character of the diet is an important factor. This metabolism plays an important part is witnessed by diabetic and cachetic pthisis atheromatosis. He accepts unqualifiedly the idea that rabbit cholesterol feeding is valid. He says, "of great significance are the investigations of Murata and Katanaka who were able to demonstrate that the artificial so-called alimentary atheromatosis which can be produced according to Kohle by lanolin feedings appears much more rapidly in castrated than in non-castrated animals". Murata was further able to demonstrate that the castration atheromatosis with lanolin feeding could be completely inhibited by simultaneous administration of thyroid gland substance. "one sees therefore how great a part the metabolism plays in the atheromatous atherosclerotic changes". (2)

Clarkson and Newbuth could not accept the idea that the intimal lesions occurring in rabbits fed cholesterol was simply due to the cholesterol and that the protein in the diet of an animal which normally gets little protein in its diet is of no consequence.

Accordingly they began an experiment on several groups of animals. They divided their rabbits into five groups. Group one served

as a control and provided normal blood cholesterol values. All of the animals used were young rabbits. Group II consisted of twenty rabbits which were fed 25Mgm of cholesterol daily over a period lasting from fifty one to two hundred eighty eight days. In no instance did the postmortem examination of the aortae of these animals show a single lesion. Group III received 113 Mgm of cholesterol daily from twelve to three hundred two days. One of these nineteen rabbits which had ingested cholesterol daily for three hundred two days showed an aorta with slight atherosclerotic lesions. Group IV consisted of twenty five rabbits which received 253 Mgm. of cholesterol daily for a period of from thirty three and two hundred forty six days and eight of the latter animals (62%) showed definite atherosclerotic lesions in their aortae. Of twelve animals in this group fed thirty three to one hundred twenty five days, eleven or 92% had normal aortae. When one compares the level of blood cholesterol with the state of the aorta one finds that four of the eight arteriosclerotic rabbits had abnormally high readings and that in the case of the other four the readings were all well within the normal.

Group V consisted of seven rabbits which were fed 507 Mgm. of cholesterol daily from forty seven to eighty six days. Five of this group had marked aortic lesions but the remainder had small lesions "that even histologically they could not be pronounced positive for atherosclerosis". This experiemnt is I believe important in that the authors fed cholesterol in capsules, thus they could be sure of a fived ingested amount.

These workers then prepared a diet containing 36% protein

as they had previously used (28) to produce atherosclerosis. A blood cholesterol determination was made just before each animal was placed on this diet and another determination was made at the end of six months, the rabbits killed and the aortae examined. The initial blood cholesterol determinations were within normal limits, the later ones showed half of the animals had blood with a very much elevated cholesterol level. The rabbits with the low or normal blood cholesterol levels showed no aortic pathology, while the four showing elevated blood cholesterol did show some intimal change.

In the discussion these authors pointed out that in this protein diet there was 10 to 15 Mgm. of cholesterol daily ingested by the rabbits. This intake of cholesterol in the protein fed animals compares in amount with Group II in none of which any aortic lesions were found. In a comparison of the results in the cholesterol fed group it was found that 75% of the rabbits in the 500 Mgm. daily cholesterol intake group developed arterosclerotic lesions with a total of 25 Gm. ingester cholesterol. By the same standards only 20% of those in the 253 Mgm. daily intake of cholesterol group developed any aortic lesions while none of the seven in the 113 Mgm. series which reached a total of 25 Gms. total cholesterol intake showed any aortic lesions. They found there was little correspondence between the blood cholesterol level and the occurrence of lesions in the aorta.

Clarkson & Newburgh reach the following conclusions. "The range of free cholesterol in the blood of rabbits, as determined by the Windaus method varied from 35 to 125 Mgm percent with a mean of 71 Mgm per 100 cubic centimeters of blood. The small amount of

cholesterol contained in the high protein diet used by us in earlier work and causing atherosclerosis does not affect the blood cholesterol . nor does it cause arterial disease. In order to produce atherosclerosis it is necessary to feed at least ten times that amount of cholesterol. In rabbits receiving such amounts both hypercholesterolemia and atherosclerosis occur but it is not possible to establish any close parrallelism between the two. High blood readings were found on rabbits with normal aortae and atherosclerotic rabbits in this series sometimes have shown a normal blood cholesterol. With still greater doses of cholesterol one finally reaches an amount which regularly produces hypercholesterolemia and atherosclerosis within a few weeks. A new series of rabbits fed the high protein diet shows that those rabbits which become atherosclerotic also develop hypercholesterolemia. We attribute this elevation of the blood cholesterol to a metabolic distrubance directly referable to the excess of protein in the diet and not to its cholesterol content". (8)

Franklin R. Nuzum, Margaret Osborne and William D. Sansum had been working with dietary changes in the rabbit for some time and were principally interested in the blood pressure changes and kidney damage they were able to produce when the animals were placed on a protein diet. (29) Later on (1926) the same workers ran a series of experiments to determine the effect of such a diet on the aorta.

A group of seven rabbits kept on a diet of liver for from three to eleven months presented extensive arterosclerosis. Three rabbits kept on this diet for less then three months did not present evidence of blood vessel change. According to the gross picture given

these animals showed the typical atherosclerotic changes; intimal thickening with yellow discoloration of the plaques. There was no evidence of spontaneous medial sclerosis in this group. The blood pressures of this group were elevated and of the group, they were highest in the animals showing the most severe atherosclerosis. In each of these animals there was also evidence of kidney injury as indicated by the casts and albumin in the urine and by an increase in the blood non-protein-nitrogen. The urines of these animals were decidedly acid the pH ranging from 5.0 to 7.0 . The carbon dioxide combining power of the blood serum was reduced.

The aortas of seven of eleven rabbits kept on a diet of oat protein with occasional greens for two years presented marked sclerosis along the thoracic and down into the abdominal aorta. The atherosclerosis was especially marked about the openings of the intercostal arteries. Medial sclerosis was present in three of the seven. The most pronounced changes were in the animals which had been on a protein diet for the longest time (2 years) the maximum blood pressures ranging between 90 and 100 MM. of mercury. The blood pressures of the controls averaged 74 MM of mercury. This group gave that same evidence of kidney injury, the urine was acid, the pH ranging from 6.0 to 6.8, the carbone dioxide combining power of the blood serum was decreased and the non-protein-nitrogen was increased.

Of twelve animals kept on soy bean protein for two years (weekly intervals greens were given) not one of the group presented true atherosclerosis. Three of this group showed medial sclerosis. This diet was given because the soy bean protein diet produces an

alkaline urine, the urine pH of this group being 9.0. This diet averaged 36% protein. This group also showed increased blood pressure and evidence of kidney damage.

The controls for this group of experiments were perfectly normal at the end of the experiment having been kept on a mixed grain and green diet. These authors therefore conclude that diets rich in cholesterol are not necessary to produce atherosclerosis in the rabbit which seems to bear out the work of Newburgh and Clarkson (30)

In an article by Moon published in 1926 there is an interesting theoretical relationship between hypertension and atheroma of the aorta. In this article he attempts to explain the process on a basis of hydrodynamics. He begins the elaboration of his theory by laying down the following premises.

"1. Fluid will flow through a collapsible tube only so long as the fluid pressure within the tube is equal to or greater than the sum of the pressure within the tube is equal to or greater than the sum of the pressures exerted externally on that collapsible tube.

2. Blood pressure in the capillaries of the vasa vasorum of an artery is less than diastolic blood pressure in that artery.

3. In hypertension the increment in capillary blood pressure is less than the increment of arterial blood pressure.

4. Circulation of blood through the capillaries of the vasa vasorum normally occurs except in the intimal portion of the artery wall. "

If the above premises are so then accordingly there is a place in the depths of the intima which gets insufficient blood from

the vasa vasorum because of the increased pressure on the vessels of the aorta due to hypertension. This same area also gets insufficient nourishment from the aorta because of its distance from it. According to this theory the etiology of the primary morbid change in atheroma, the mucoid degeneration of the intima is an ischemia.

This idea of Moon's although he recognizes the purely theoretical nature of it does according to the author explain various observed facts in connection with atherosclerosis, some of these are, the consistent early occurrence of atheroma at the mouths of the intercostal arteries. In applying this principle here atheroma takes place in this location because the intima about the orifice of these vessels is subjected to the increased blood pressure both in the aorta and the tributary artery. This would also apply to the openings of the abdominal vessels and the bifurcation of the aorta to form the iliacs.(27)

Duguid is inclined to the idea that the cause of the earliest change in atheroma is a tearing of the intima from the media as the intima is thrown into folds as the pulse wave passes downward, this he believes to be the cause of the edema and hyaline change which occurs at the base of the intima. Following this there is a deposition of lipid material in the intima and a compensatory fibrosis. Anything which will accentuate the pulse wave will increase the response in the intima, the most important factor in this connection is hyperpiesis. (10)

In a report given by Joslin he discusses the etiology of arteriosclerosis in the diabetic. He believes that a protein element in the etiology of diabetes should be assumed. "General acceptance of



the statement can be expected that an excessive protein diet is not only harmful to the diabetic but to normal individuals and tends to produce directly or indirectly arteriosclerosis". He offers little in proof for this statement. Of carbohydrate he presumes that hyperglycemia might be an important factor but he grants that this is not proven by any experimental work. Acidosis is thought to be an important element in arteriosclerosis but here again no experimental observations are quoted nor are any available. In this connection he thinks that the ketogenic diet given to epileptics may in time develop more about acidosis as a factor. Fat and cholesterol are thought by Joslin to be very important factors. He lists several articles which when included in a diet cause of hypercholesterolemia in diabetics. He believes that hypercholesterolemia and hyperlipemia go hand in hand.

(16)

Sweeney and Smith (1930) used adult albino rats as animals in which to produce experimental arteriosclerosis. In the first group of animals one of the experimental group was given high salt diets consisting of sodium bicarbonate and sodium chloride. At the end of several months the findings were negative, there was no apparent arterial change. The use of Staphylococcus suspensions in saline solution also produced negative results as far as morbid change in the arteries was concerned. A third group received viosterol 1.5 to 2.0 cubic centimeters in their daily diet this caused the death of one animal after fifty five days of experiment, one after sixty seven days and the third was killed after seventy four days. X-Ray pictures taken late in the experiment showed a dark shadow along the route of the aorta. Microscopic examin-

ation of the layers of the aorta revealed the intima to be the chief site of deposition with involvement of the media in the most extensively and thickly calcified areas. "The amount of calcification was in direct proportion to the amount of viosterol which had been consumed by the animals".

One animal was given the same treatment and then allowed to rest for a month without treatment then killed, examined and the same results were found. It was then attempted to decalcify the aortas by the use of parathyroid hormone but without results. It was then concluded that the consumption of approximately 50 cubic centimeters of viosterol over a twenty five day period is sufficient to cause definite arteriosclerosis in the adult rats. (38)

That the secretion of the posterior lobe of the pituitary by virtue of its effect on fat and carbohydrate metabolism might also play a part in atherosclerosis was the idea behind the work of Moehlig and Osis (1930). They used four groups of rabbits, one a control, one fed a diet rich in cottonseed oil and lanolin, one fed on the same diet as that given the second group and in addition receiving hypodermically 1.0 cubic centimeters of posterior pituitary extract, the last group was carried on a control diet but received the pituitary extract. The two groups receiving the high fat diet had an abrupt and large rise in the blood cholesterol, it averaging about 440 Mgm. per cent. Both of these groups showed intimal thickening with a deposition of cholesterol in the intima, the process being more severe in those animals which also received the posterior pituitary extract in addition to the high fat diets. Of the fourth group or the one which received

the basal diet and the pituitary extract six of the ten animals showed normal aortas. Four of this last group showed changes suggestive of early arteriosclerosis. As there is no description of picture of the lesions in this last group one cannot be sure just what these changes "suggestive of early arteriosclerosis" means. One thing of value in this work might be that the pituitrin in addition to the high fat diet might make the aortic lesions more severe in a shorter time. (26)

In following up the work of artificially produce arterial lesions in experimental animals by the introduction of vitamins the work of Vandever is of note. He used young rabbits and gave them tri-weekly subcutaneous injections of ergosterol .5 cubic centimeters per kilogram of body weight, one cubic centimeter of which was equal to 10,000 D units, he believes the lesions so produced to always lie in the media. The earliest lesion he was able to observe was a laying down of calcareous particles along the elastic fibers just outside the internal elastic lamina. He found this calcareous deposits in the media before there was any demonstrable degenerative changes. The intima was practically never involved and when it was the location of such that there could be no relation between the intimal lesions and those of the media. The favorite sites geographically were the arch of the aorta particularly the outside of the arch and less frequently on the inside of the arch. He found no lipid deposits in the media or the intima at any time during the experiment that were of any consequence. In this connection it might be noted that the animals as a whole when autopsied were practically devoid of any gross fat. Any malformations of the intima which did occur he was able to demonstrate that they were

due to calcification of the underlying media. One of the animals which received not only the ergosterol but also calcium gluconate showed a perfectly normal aorta. (41)

Benson and others after observing severe atherosclerotic lesions in a young girl who had died of gangrenous stomatitis decided to try some experiments to determine the effect of cultures of bacteria inoculated intravenously into rabbits. In many instances in which death was due to coronary arteries when the blood stream in general was negative to culture. Most of their experiments were conducted with organisms obtained in this way from human autopsy material.

A litter of seven rabbits was inoculated intravenously with streptococci obtained from a case of coronary thrombosis. They were given a massive dose, observed for a week, then killed and examination of the aorta was negative.

Of a second litter of seven rabbits, five received seven inoculations each from a culture of "Streptococcus fecalis" over a period of five and half months. Rabbit number five in addition to its inoculations had thirty three cholesterol feedings of 0.5 Gm. each, rabbit number six had only the thirty three cholesterol feedings, rabbit number seven served as a control. All were killed at the end of six months. On examination rabbit number five was the only one which showed any gross changes in the aorta. The ascending aorta of this animal contained thickened yellowish ulcer like plaques in which the intima was considerably raised. There was also a more diffuse irregular thickening of the whole ascending aorta and to a lesser extent the descending aorta. Microscopic examination disclosed a decided thickening and

irregularity of the intima with considerable fibrous increase and scarring with obscuring of the internal elastic lamina. Fibroblasts were scattered throughout the intima and a few vacuoles were present. The intima also contained elongated streaks of blue staining material. Sudan III and hematoxylin staining disclosed a diffuse infiltration by small globules of lipin throughout the intima.

Litter three consisted of two young rabbits without controls. Each animal received three inoculations of "Streptococcus fecalis" in a period of two months, at autopsy one showed small arteriosclerotic patches in the ascending aorta, one did not.

Litter four consisted of six rabbits two of which were used as controls. Three animals received two inoculations each of Streptococci and another received four. The inoculated animals, were also given a subcutaneous implanation of 2 Cm. of aseptic rabbit aorta in the hope of inducing absorbtion of enough aortic substance to influence the electivity of the injected organism for aortic tissue. The organisms used had been repeatedly cultivated over a period of many days in dextrose infusion broth containing a protion of sterile rabbit aorta. One rabbit died a month after inoculation with arterosclerotic plaques in the aorta, the other five rabbits including the controls lived from one to three months and showed no gross lesions.

Litter five consisted of five animals which received two inoculations. Four months later at autopsy none of these animals showed any gross lesions.

Litter six consisted of six animals three of which were inoculated twenty seven times with Streptococci, two had one hundred

twenty seven and one hundred thirty one cholesterol feedings one with the Streptococci and one without inoculations. The results were in no wise conclusive. One animal which received the bacteria alone showed no lesions, the animal which received the cholesterol alone showed no lesions, two of those animals receiving the bacteria along showed some aortic lesions, the animal which had been given both the cholesterol and the bacteria also had aortic lesions.

Some experiments along the same line were done by the same workers along the same line using *Macacus rhesus* but they were few in number and inconclusive. As a result of these experiments it was concluded that atheroma of the aorta could be produced by the intravenous inoculation of Streptococci. They were of the opinion that feeding of cholesterol to these animals in addition to the bacterial inoculations induced more severe lesions. (5)

Turner, in 1933 became interested in the prevention of cholesterol, induced atherosclerosis in rabbit. He set out to determine the effects of whole thyroid substance and potassium iodide. He divided a group of animals into two groups, an experimental and control group. All of these rabbits were between four and five months old. The cholesterol was administered by the mixing of one gram of the crystals in the food three times a week. By this method it was possible to induce atheromatous changes in the aortas of one hundred percent of the animals in from eighty seven to one hundred fourteen days.

The first experiment consisted of fourteen rabbits which were on the cholesterol diet. On examination all of these animals showed atheromatous change. The blood cholesterol averaged 520 Mgm. percent.

The oxygen consumption per gram per hour was 0.472 cubic centimeters.

The second experiment consisted of nineteen animals which in addition to the cholesterol in the diet also had 0.4 Gm. of dried thyroid substance mixed with the food three times a week. At post-mortem only two of the animals showed slight and moderate atheromatous change. The blood cholesterol averaged 178 Mgm. percent. The oxygen consumption per gram per hour was 0.799 cubic centimeters. The rabbits which did show the atheromatous change in the aorta had a hypercholesterolemia; one had a blood cholesterol level of 291 Mgm. percent, the other 261 Mgm. percent.

The third experiment consisted of eleven rabbits. In addition to the cholesterol in the food they received 1.2 Mgm. of thyroxin injected subcutaneously once a week. In this experiment six of the rabbits died in forty four to eighty days. Of the entire eleven, three showed marked atheromatous change of the aorta, four showed moderate change, one slight change and three showed no change. The average blood cholesterol level during this period was 399 Mgm. percent. The oxygen consumption was 0.626 per gram per hour. All of the animals showing atheromatous change had a hypercholesterolemia.

In the fourth experiment seven animals were used as controls. Twelve rabbits were given a gram of potassium iodide in aqueous solution three times a week in addition to the cholesterol diet. In only one animal of this group was there any atheromatous change, in this animal it was marked. The average cholesterolemia was 183 Mgm. percent. Another experiment was tried in which potassium bromide was substituted for potassium iodide. In this series of seven rabbits only one showed

any atheromatous change. The average cholesterolemia of this group was 428 Mgm. percent. In another experiment along this same line potassium chloride was substituted for the iodide. Of this group of ten animals only one failed to show aortic lesions and this animal had a normal blood cholesterol. The blood cholesterol of the entire group averaged 429 Mgm. percent.

From these experiments it seems obvious that there is a direct relationship between the blood cholesterol level and the degree of aortic pathology. Thyroid substance is a better inhibitor of atheromatous change than is crystalline thyroxine. Both thyroxine and thyroid substance increase the basal metabolic rate, this the iodides do not do. Apparently ionic iodides and bromides have the same effect as thyroid gland and thyroxine, chlorides do not have this capacity. (39)

With the relation between the iodides, thyroxine thyroid gland and the bromides and experimental atheromatous change established, Turner and Khayat conducted some experiments to determine the effect that thyroidectomy might have on experimental atherosclerosis. In this experiment a complete thyroidectomy was done, the rabbits were fed a stock food of oats, alfalfa, molasses mixture and fresh vegetables. The animals were likely to eat poorly for a short time following the operation and lost some weight for a week or two. Following this they ate well and gained back the lost weight but did not become obese.

Group one consisted of the control rabbits, seven of which had been thyroidectomized and five normal ones. These animals were given the regular diet without the cholesterol. Of the normal animals there was no atherosclerosis, the blood cholesterol level varied from



151 Mgm. percent to 98 Mgm. percent. All were killed and examined on the one hundred tenth day of the experiment. Of the thyroidectomized animals none showed any atherosclerosis, five of these were killed on the one hundred tenth day of the experiment, one died on the seventy fourth day and one was transferred. The blood cholesterol level varied from 156 to 97 Mgm. percent.

The third group consisted of five animals which received one gram of cholesterol in the diet three times a week. None of these animals had been thyroidectomized. All of these animals except one showed aortic lesions and that one died on the forty second day of the experiment. Blood cholesterol varied from 107 Mgm. percent early in the experiment to 536 Mgm. percent later in the course, showing in general a progressive rise.

The fourth group consisted of seven thyroidectomized animals fed on the cholesterol diet. Of the four who lived the full one hundred ten days of the experiment all showed aortic lesions. The blood cholesterol rose progressively from 130 Mgm. percent to 606 Mgm. percent.

Group five consisted of six normal rabbits fed cholesterol and potassium iodide. All were killed on the one hundred and tenth day of the experiment and none showed aortic lesions. The average cholesterolemia varied from 146 to 218 Mgm. percent.

Group six consisted of eight thyroidectomized animals who were fed cholesterol and potassium iodide. Of the eight animals only three lived to the one hundred and tenth day all of which showed some aortic lesions as did one which died on the fifty sixth day. The others either were removed from this group or died early and did not show any

aortic lesions. The cholesterolemia varied from 117 to 582 Mgm. percent it becoming progressively higher as the experiment progressed. The two animals removed from this group on the thirith and the forty third day of the experiment after showing a hypercholesterolemia were then put on a control diet for one hundred ten days during which time no significant rise in blood cholesterol occurred. At autopsy there were no gross aortic lesions, each animal showed a mass of regenerated thyroid tissue as large if not larger than that of a normal thyroid. From these experiments it would appear that the potassium iodide exerts its inhibitory effect through the thyroid. (40)

In an article published by Leary in 1934 he reviews the subject of experimental atherosclerosis particularly from a standpoint of comparison with the same lesion occurring spontaneously in the human coronary arteries. He induced the atheromatous condition of coronary arteries of the rabbit by the ingestion cholesterol, this he gave to the experimental animals by catheter. He found that few of the animals would tolerate more than one gram daily. It is interesting to note here that he at that time thought that the earliest lesion seen in the experimental animals was the imbibtion of or at least the collection of cholesterol under the endothelium. In a later paper he changes this somewhat. The similarity is very marked when one takes into consideration that the lesions seen in the human are much later than those seen in the experimental animal.

He concludes that the lesions of human atherosclerosis can be produced in the rabbit by the feeding of cholesterol, that the fibrosis that is characteristic of human coronary lesions in the young

is the characteristic lesion in cholesterol atherosclerosis in young rabbits. Fibrosis is therefore a reaction of youth and not of species. He is of the opinion that the pathogenesis can be studied to much greater advantage in the rabbit than in the human because the lesion progresses so slowly in the human. He believes that there is much data to support the contention that atherosclerosis in the human is of cholesterol etiology. He cites several references which would seem to indicate that while cholesterol forms a part of every animal cell still there is none of it synthesized within the body, that the only source of cholesterol that man has is from the outside. He states, "man is the only animal that ingests milk and eggs throughout its life time. Man is also the only animal as far as is known which dies in early life from coronary sclerosis, and which acquires atherosclerosis almost universally in advanced life". He believes that the recent high fat diets given to diabetics was the cause of the large percentage of arterosclerosis occurring in diabetics. (22)

Rosenthal in a recent article (1934) uses the statistical method in his study of human atherosclerosis. His material consisted of five hundred autopsies performed at Cook County Hospital in Chicago. All of these bodies were over twenty five years of age. His work properly speaking is not in the field of experimental atherosclerosis but he develops some ideas which have some bearing on this subject.

He begins by criticising the present method of classification of atherosclerosis; what might be called mild atheromatous change in one place which be called moderate or slight by others. In this case the aortae were removed by cutting as close to the valves as possible,

cutting the branches off flush with the aortic wall, the adventitia was carefully stripped away and the whole aorta examined for fat. It was found by this method that the total amount of extractable fat was directly proportional to the degree of atherosclerosis, that the lipid was mainly composed of free cholesterol and its ester in atherosclerotic aortas the relationship between these elements was constant, that is twenty five to fifty seven percent respectively. He concluded by this summary that the aortic fat increased with age and when a sufficient number of cases were had in a homogenous group the ascent approached a straight line. From this study he evolved what he calls a fat angle of the aorta or in short a F.A.A., that is; the tangent of the angle is equal to the grams of fat in the aorta times one hundred minus two; divided by the age of the body minus fifteen. This is not only the fat angle of the aorta but is also the inclination of atherosclerosis. By this method the angle of 19.3 degrees indicated a smooth aorta, an angle of 48.1 degrees a slight to moderate atherosclerosis, an angle of 71.4 degrees a moderate to severe atherosclerosis.

In Rosenthals discussion of exogenous cholesterol as a factor in atherosclerosis he cites some statistics which would seem to indicate that in general the higher the dietary protein the greater the incidence of atherosclerosis and hypertension. A notable exception to this is the Eskimo. He then tries with indifferent success to fit the (fat angle of the aorta) to the negro in the north and the negro in the south where the standard of living is lower and less protein in the diet and to show that where the protein in the diet has increased the atherosclerosis has also increased. (34)

Page and Bernhard studied the effects of organic iodide on cholesterol-induced atherosclerosis. The animals were divided into two groups. One received 200 Mgm. daily of cholesterol dissolved in warm olive oil six days a week. Another group in addition to this received the di-iodide of ricinsterolic acid. The experiment lasted from one hundred nine to one hundred eighty days. All of the rabbits fed cholesterol showed an extensive deposit of cholesterol in the intima of the aorta while those animals which received in addition the di-iodide of ricinsterolic acid showed no deposition of cholesterol in the intima. Persistent lipemia developed in both groups of animals but the lipemia was more marked in the group which was fed the organic iodide. (31)

In determining the effect of alcohol on cholesterol induced atherosclerosis Eberhard found that in animals fed both cholesterol and alcohol that the cholesterol level of the blood rose more rapidly and to a higher level than it did in animals receiving cholesterol alone, but that the deposition in the tissue of the liver and aorta occurred in the inverse ratio. It was also found that a group of rabbits that received only alcohol in addition to a basal diet of grain showed a slightly higher blood cholesterol and a definitely lower cholesterol content of the aorta than the control group. (11)

Jobling and Meeker made an unsuccessful attempt to accelerate and increase the development of cholesterol lesions in the aorta of the rabbit by the intravenous injections of Streptococcus toxin, production of artificial fever, ammonium chloride feeding, intravenous injections of peptone, induction of anaphylactic shock,

and the intravenous injection of uric acid. One of these procedures were done simultaneously with the feeding of cholesterol in each experimental group. No affect at all could be shown on the blood vessels of the cat when fed cholesterol and in addition were inoculated with peptone and histamine intravenously or were fed ammonium chloride.(15)

Short, Bruger and Jaffe studied the effects of acetonemia on the aorta of rats. The animals were divided into various groups, one serving as a control, one receiving 500 Mgm. of aceto-acetic acid, daily, one 1,500 and one 2,500 Mgm. of aceto-acetic acid in their diet daily. The experiment lasted six months, at this time only one of the experimental animals showed any intimal lesions and these were in the femoral artery. To make sure that an acetonemia was produced the expired air was tested and found positive for actene. While the authors admitted that they had not produced the ketonemic state such as exists in diabetes they concluded that acetonemia alone would not produce any intimal change in the rate. (37)

To Leary we are indebted for the best account of the pathogenesis of atherosclerosis to date. In a recent article of his he goes over the lesion and describes the appearance of it in detail from the time of the early lesion in youth to the adult lesion. In this article he states that the earliest lesion that he has observed is in the ground substance of the intimal connective tissue, this change he refers to as a mucoid degeneration. The next change is the occurrence of finely granular lipoid substance in the interstices of the connective tissue of the intima. At this time this fatty material is isotropic. Next in order globular microphages occur in the intima and become fill-

ed with fat which then becomes anisotropic. This change in the refraction of the lipoid substance he believes is due to the formation of cholesterol esters from the lipoid which originally was found in the intercellular spaces of the intima. Following this there appears a spindle shaped cell in the deeper layers of the intima which become filled with lipoid material and in which the fat becomes isotropic again. Up to this point the reaction for all of the age groups are about the same except the rapidity of the severity with which it may take place. In the younger age groups from this point on the lesion may be removed with little or no scarring. This, Leary believes to be due to these spindle shaped cells metabolizing the cholesterol. He called these cells the fibrolipophages. He does believe that some of the cholesterol is removed from the lesion by the migration of these cells into the lymphatics of the aorta, having found some of them in the lymph channels of the media and the adventitia adjacent to a lesion which he considered to be undergoing resolution. The important thing about the process in youth is that the lesion undergoes complete resolution with little or no scarring. The fibrolipophages become fibrocytes of the intima again. As we progress into the lesion as it occurs in the middle age there are some differences principally in the later phases of the lesion. This Leary believed to be due to two factors; the individual as he grows older has not the ability to metabolize cholesterol to the same extent as he did when younger, there is a greater tendency for the connective tissue to form collagen and this when actual scarring occurs interferes with the nourishment of the lower levels of the intima which must obtain all of its nourishment from imbibition from the lumen of the

artery. Thus when this lesion occurs in the older individual there is a greater tendency to form the atheromatous ulcer. In this connection Leary does not like the word ulcer and substituted the word (atherocheuma) which he says meets all of the requirements which the term atheromatous ulcer does not. In the aged this same tendency is manifest only to a greater extent.

The whole article is well done and well illustrated with photomicrographs. There is no question of the pathogenesis as presented in this paper. There are some inferences which are not well founded however. He infers that because the ova of many animals is rich in cholesterol that the young individual has greater capacity to metabolize it and thus explains the cells he finds deep in the intima of the lesions which in appearance resemble the fibroblast as being the ones which metabolize the cholesterol.

In his discussion of the factors which influence the deposit of cholesterol in the arteries, he is of the opinion that a high cholesterol content of the diet is an important element. He discounts the fact that in bodies containing quite severe atherosclerosis there has been found a normal or below normal cholesterolemia as not being significant because he states the cholesterol level of the blood undergoes rapid and great fluctuations and the atheromatous lesions occur only with long continued hypercholesterolemia. He reviews of work of Murata and Kataoka and Turner and recognises the part that iodine must play in the development of these lesions. "Based on the observation that the cholesterol content of the blood tends to be low in subjects with hyperthyroidism and high in those with myxedema, Turner's



experiments established the potency of feeding of whole thyroid in checking the arterial deposit of dietary cholesterol and the inefficacy of iodine in this respect in thyroidectomized rabbits".

Of the early mucoid degeneration Leary says, "When one considers the association of atherosclerosis with myxedema, the report of advanced atherosclerosis in cachexia thyreopriva following total ablation of the gland in the early days of surgery of the thyroid gland, the occurrence of atherosclerosis after thyroidectomy in animals, the power of thyroid feeding to prevent atherosclerosis in cholesterol fed animals, the efficiency in this respect of the administration of iodine in the presence of the thyroid gland, and its inefficiency after thyroidectomy one is led to believe that the mucoid change in the subendothelial layer of the intima may be the result of some form of insufficiency of the thyroid gland that is not marked enough to give rise to the clinical disease myxedema."

Age he does not consider to be significant because of the occurrence of atheromatous change in the young. He does point out the connection between the diminution of the function of the thyroid gland with age as fitting into his idea of less efficient cholesterol metabolism. He considers stress as being a factor because of the fact that the earliest lesions always occur at the orifices of the intercostal arteries.

Infection and injury of the intima are accepted by Leary as being probable causative factors but he discounts this approach because of the improbability of it operating in man such as it has been used in experimental animals.

Toxic factors he rules out completely as being too ill defined. He says that he was unable to confirm the work of Newburgh and Clarkson, consequently he does not believe that toxicity due to feeding a high protein diet is a factor. (23)

Under the heading "Evidence that Cholesterol is the Etiologic Agent" Leary says the following.

- "1. (a) Cholesterol is constantly present in the lesions of the disease.
- (b) It can be isolated from the lesions.
- (c) When fed in purity to rabbits, it will give rise to the lesions of the disease.
- (d) It is constantly present in the experimental lesions.
- (d) No other known agency will produce even suggestive results.

Were this a question of a disease of bacterial causation cholesterol would fulfill all requirements as the specific etiological agent.

2. It has been shown in a preceding paper that the variation in the character of the aortic lesions in atherosclerosis is dependent on the relative ability of phagocytic cells to metabolize cholesterol. In other words, human atherosclerotic lesions apart from the initial mucoid change are produced because of the presence of cholesterol and the varied appearances these lesions present are the results of differences in the reaction to cholesterol of the tissue in which that substance lies, differences which depend largely on the age of the subject or of the lesions.

3. In connection with no other disease has there been such wide spread human experimentation in pathogenesis as was carried out by the feeding of diabetic diets high in fat rich in cholesterol in the decade

1920 to 1930. The results were as definite as these obtained in the experimental rabbit. (24)

Since Hodgson had aortic lesions analyzed in 1815 and the analysis by Lassaigne somewhat later, (9) Baldaug (1906) at the beginning of what may be called the modern era (4) and up to the present time biological chemistry has placed an important part in the research concerned with atherosclerosis. Most of the earlier work was done on calcified arteries, the later work has been to a large extent concerned with the lipid content of the blood, the arteries and with cholesterol metabolism. Bloor (7) and Bloor and Yasuda (42) in this country are responsible for working out the best methods of cholesterol analytical methods.

In regard to analysis of cholesterol in the atherosclerotic lesions different authors are at a variance. In the work of Rosenthal (34) formerly referred to he maintained that the only accurate method of classification of lesions was by chemical analysis. Zeek (43) on the other hand believes that aortas which appear alike microscopically are alike chemically. In a series of studies made by Kirk, Page and Van Slyke it was concluded that the averages for total blood cholesterol in the different age groups do not differ significantly. (32) Haythorn and others found that there is a consistent increase in the calcium contained in the aorta with age, what this might have to do with atherosclerosis is not suggested. (12) (13)

Bills, in a comprehensive review of the literature on the biochemistry of cholesterol made in 1935 was unable to assign any definite metabolic role to cholesterol. He says, "the following

suggested roles seem important enough to be mentioned in review. By force to repeated citation, some of these have come to be regarded as established, but the facts do not warrant such categorical conclusions". A physiological relation between the sterols and the physiologically important substances which are chemically related to them is a possibility of intriguing interest. Cholesterol may have a conditioning action on the skin, hair and feathers. Cholesterol may function as a conveyer in the absorption of fats. Cholesterol may function as an insulating medium for the myelin sheaths. Cholesterol may have some function in regard to cell permeability. It has been inferred that cholesterol may function as a food supply for the developing ovum. Cholesterol may function as a detoxifying substance. Cholesterol may have some part in the prevention of anaphylactic shock. While the above mentioned possibilities may occur, Bills in his analysis of the literature failed to find any conclusive proof for any of them. (6)

When experimental atherosclerosis was first produced there was considerable discussion as to whether or not the lesion was similar to that occurring spontaneously in man. There was even some confusion as to the various types of arterosclerosis and their relationship to each other. As early as 1906 Klotz was in disagreement with Thoma who maintained there was a direct relationship between the sclerosis of the medial coat of the arteries and atherosclerosis. (18) Klotz by his experiments was instrumental in proving that no such relationship existed. (18) Since that time practically all workers have been in agreement on this point. That the cholesterol-induced lesions of the intima of the rabbit aorta were not similar to those occurring

spontaneously in the human has been and still is a live issue. The work of Newburgh and Clarkson raises this objection. (8) It seems unlikely though that after the work of Leary (22)(23) (24) that this objection is a valid one and that the experimental method is such that one must conclude the increased ingestion of cholesterol or the persistent hypercholesterolemia is one of the main etiological factors. (24) This is contrary to the work of Lande who on a numerous series of postmortem examinations on bodies whose death was caused by trauma could find no relationship between the incidence of atherosclerosis and the blood cholesterol level. (21) The work of Page and Bernhard would also question the validity of a hypercholesterolemia as being the important etiological factor but in this work additional elements entered the picture. (31) The results with other methods of experimental atherosclerosis; trauma, acetonuria, protein diet, intravenous injections of bacterial cultures and toxins are for the most part either insufficient or too inconsistent to be accepted as important etiological factors in place of cholesterol.

According to Aschoff, Virchow was the author of the idea that edema and hyalinization of the intima preceded the deposition of cholesterol in the atherosclerotic lesions. This has been quite generally accepted as being true but the mechanism by which it was produced has varied somewhat. Many of the earlier workers and some of the present one are of the opinion that it is produced by some toxin the nature of which is unknown. (19) Duguid was of the opinion that it was due to the stretching of the artery which tore the lower layers of the intima loose from the internal elastic lamina, he also though

that hyperpiesis accentuated this condition. (10) Shapiro proposed the idea that stagnation of the blood in the smaller arteries was the important factor in the production of edema and hyaline change in the intima. (36) He did not however try to fit this principle to the aorta. Leary believes after a review of the more recent work of the inhibition of atherosclerotic change by the use of iodides that this early intimal change is bound up with deficient thyroid metabolism; that this represents a very early myxedematous change. (23)

The pathogenesis of the experimental lesion is quite definitely known. Beginning with the description of Klotz (17)(18) and ending with the elaborate description of Leary's. (23) It is now quite generally accepted that the different picture one sees in the human is simply due not to a different etiology but to the different reaction of a older tissue to the same noxious influence.

The metabolism involved in atherosclerosis is not very well understood, because there is so little known about the metabolism of cholesterol. The work of various men in inhibiting atherosclerosis by the use of iodides, thyroid, thyroxine and alcohol may in the future cast some light not only on the mechanism of atherosclerotic change but also on the metabolism of cholesterol.

The etiology of atherosclerotic change is in some way bound up with either intake of cholesterol or persistent high blood cholesterol levels. The earliest morbid change in this process is an edema and hyaline change of the connective tissues of the intima which may be due to some alteration of thyroid metabolism. The pathogenesis from this point on is well known. There is nothing definite known about the

metabolism of cholesterol, this fact seems to be the greatest challenge in the field of experimental atherosclerosis at the present time.

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