Experimental Atherosclerosis

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THIS presentation is concerned exclusively with one variety of arteriosclerosis, atherosclerosis. This subject delineation is not an arbitrary or artificial one. The term arteriosclerosis is a generic one. It refers to several distinct pathologic entities producing thickening of the vessel wall, embracing such entities as atherosclerosis, Mönckeberg’s sclerosis, arteriolosclerosis, and hyperplastic arteriosclerosis. Awareness of this fact is absolutely essential for progress in this field. Recent research reinforces the conclusion that these are different entities. The attention of investigators is correctly focused today on the problem of the etiology and pathogenesis of atherosclerosis, since this is the lesion among the arterioscleroses which is overwhelmingly responsible for morbidity and mortality in man.

Until very recently atherosclerosis was a step-child problem of medical research commanding very limited resources of personnel, equipment, plant and money. This situation, only partially improved upon at present, is a consequence of several circumstances. Among them, one of the most important is a fundamentally erroneous concept developed in the medical profession itself—the senescence “theory” of the genesis of the arterioscleroses. This “theory” maintains that the arterioscleroses are inevitable results of physiologic aging processes. This “theory” likewise regards the specific entity atherosclerosis as such an unavoidable process of aging. The stagnating influence of this “theory” upon medical research has been overwhelming. It engendered an atmosphere of helplessness and hopelessness that was for many years a serious brake on all investigation.

This “theory” is a patently erroneous dogma. Certainly today overwhelming clinical evidence exists that atherosclerosis occurs in some very young people and is absent in some very old people. This indisputable evidence is alone sufficient to demonstrate that atherosclerosis cannot be an inevitable by-product of senescence. Rather, senescence and athero-genesis are two distinct and not necessarily related processes. Data from the experimental laboratory reinforce these conclusions of clinical research. Moreover, these data supply considerable support for the concept that atherogenesis is consequent upon an alteration in lipid metabolism, particularly in cholesterol metabolism.

It is upon the basis of this cholesterol concept of atherogenesis that fruitful research in this field is proceeding apace in a number of laboratories. The basic tenet of this concept may be simply stated: without an altered lipid-cholesterol metabolism little or no atherosclerosis will develop regardless of any other alterations in the arterial wall, including senescent changes. Obviously, if atherosclerotic lesions are the result—or even only in part the result—of altered lipid-cholesterol metabolism, then they are not inevitable. The whole foundation of the senescence theory is rendered untenable. The possibility, nay inevitability, presents itself that preventing or reversing the altered lipid-cholesterol metabolism will eliminate atherosclerosis. Thus, a hopeless situation is changed to one full of promise. Research in this field, by attacking the problem of the detailed interrelations

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between altered lipid-cholesterol metabolism and atherogenesis, can look forward to forging the scientific preconditions for eliminating atherosclerosis as a disease.

Evidence from Man

The cholesterol concept of atherosclerosis, the basis of almost all experimental work in this field at the present time, has an extensive foundation in clinical research. At least four sources have furnished supporting evidence for it: histopathology, biochemical pathology, ethnopathology and clinical pathology. A review of the extensive data from these sources is beyond the scope of this presentation. To summarize briefly the essential facts from these sources, histo- and chemopathology early revealed that extensive lipid and cholesterol deposition is a hallmark of the atherosclerotic lesion. From the ethnopathologic viewpoint, comparative studies of vascular lesions among different peoples, including Costa Ricans, Okinawans, Chinese, Japanese, American and African Negroes, Eskimos and others, indicate that significant differences exist among peoples in incidence and severity of atherosclerosis. These differences appear to stem from culturally conditioned variations in nutrition and diet, rather than from racial, climatic or other influences. Specifically, a high correlation is frequently demonstrable between presence or absence of atherosclerosis and luxus or paucity of foods high in animal fat and cholesterol. Data accumulated in European countries during and after World Wars I and II lend further weight to the concept that there is a relationship of diet to atherogenesis. From these multiple studies the following tentative conservative conclusion appears justified: atherosclerosis is generally more frequent and severe in well nourished, particularly overnourished, people subsisting on diets rich in animal fat. Finally, from the clinicopathologic viewpoint, it is abundantly clear that in a number of disease states characterized by alterations in cholesterol metabolism and a prolonged hypercholesteremic phase, among them hypothyroidism, the nephrotic syndrome, essential familial xanthomatosis, biliary obstruction, and diabetes mellitus, premature severe atherosclerosis is inordinately frequent. Moreover, evidence has recently accumulated indicating that patients with coronary atherosclerosis without gross hypercholesterolemia have subtle alterations in cholesterol metabolism and a “xanthomatous tendency.” The suggestion, based on these data, of a close association between alterations in cholesterol metabolism and atherogenesis is further reinforced by the recent work of Gofman and his colleagues demonstrating abnormalities in circulating cholesterol-bearing lipoproteins in association with clinical atherosclerosis. This, in brief, constitutes the clinicopathologic background and foundation for experimental atherosclerosis.

Experimental Atherosclerosis

History

Experimental atherosclerosis was first successfully induced in animals in the period 1908 to 1912. Prior to that time, all experimental attempts to reproduce the atherosclerotic lesion of man had failed, although arterial lesions had been produced by a number of procedures in animals (such as treatment with drugs, pathogenic bacteria, toxins). However, the resultant changes in the vessels resembled the Mönckeberg type of human arteriosclerosis (medial calcnosis, senile arteriosclerosis), rather than atherosclerosis. In 1908 a group of investigators working in St. Petersburg studied the effects of various dietary regimens on vascular pathology. They observed that rabbits fed diets containing meat, milk or eggs developed atherosclerosis. It was soon demonstrated, particularly by Anitschkow, that the biochemical factor responsible for these lesions was cholesterol. Conclusive proof was advanced that this sterol was the atherogenic stimulus. Experimental atherosclerosis as a definitive field of research endeavor dates from these discoveries. It is noteworthy that to this day experimental atherosclerosis has not been successfully produced by any other means than cholesterol administration (except avian stilbestrol-induced atherosclerosis, a lesion secondary to endogenous endocrine-stimulated hypercholesteremic hyperlipemia).
The Chick as an Experimental Animal

About 10 years ago this department became interested in experimental atherosclerosis. Our studies were carried on in the past decade principally in the chick. Our choice of this experimental animal was not fortuitous. Rather, it was based on a purposeful attempt to proceed along lines obviating criticisms leveled by many at the use of the rabbit for such atherosclerosis research. The rabbit is a herbivorous species not normally ingesting cholesterol. Moreover, on its usual diet it does not naturally develop vascular lesions of the atherosclerotic variety. Finally, until the last decade experimentalists had experienced only limited and indifferent success in attempts to induce gross atherosclerotic lesions by cholesterol feeding in species other than the rabbit. All these facts led many workers to question seriously the relevance of cholesterol-induced atherosclerosis in the rabbit for an understanding of the pathogenesis of the human disease. It was upon this basis that we sought to find a more suitable experimental animal. The chick was finally selected after a careful survey of the extant literature.

The chick, like man, is omnivorous, and develops atherosclerotic lesions of the great vessels spontaneously. In early studies it was quickly demonstrated that cholesterol feeding led to hypercholesteremia and eventually to atherogenesis in chicks. Such cholesterol-fed birds had gross atherosclerosis not only of the aorta, but also of the major systemic vessels, including the coronary arteries. These cholesterol-induced lesions were readily distinguishable from spontaneous lesions. Further, cholesterol was readily shown to be the decisive atherogenic stimulus in chicks, as in rabbits.

These initial studies demonstrated that the chick is of unique utility in offering two types of lesions for experimental study, the spontaneous and the cholesterol-induced. In 1946 the horizon and methodology of experimental atherosclerosis in the chick were further broadened. In that year Chaikoff and his associates showed that atherosclerosis occurred in cockerels having a prolonged endogenous hyperlipemia induced by implantation of estrogenic material. Horlick and Katz confirmed this finding and extended it by demonstrating that the lesions in stilbestrol-treated chicks were definitely not of the spontaneous type. Thus, a third experimental lesion in chicks became available for study. This avian species possesses, then, a unique versatility which further enhances its utility in atherosclerosis research.

Morphology of Atherosclerosis in the Chick:

In several publications from this department, a detailed description has been given of the morphology of spontaneous, cholesterol-induced and stilbestrol-induced atherosclerosis in cockerels. A general review of the pathology of these lesions is beyond the scope of this presentation. However, several specific points may be noted: (1) Detailed microscopic studies indicate that throughout the arterial tree of stilbestrol-treated or cholesterol-fed chicks the primary lesion of experimental atherosclerosis is the foam cell intimal cushion. This lesion, so-called pure atheroma, is almost certainly the first stage of atherogenesis. The morphologic patterns of more advanced lesions are apparently the result of evolutionary pathologic processes secondary to atheroma. (2) It is possible by prolonged cholesterol feeding to induce in chicks the spectrum of atherosclerotic changes seen in human lesions including foam cell plaques, necrosis and atheromatous abscesses, fibrosis and hyalinization, calcification and cartilaginous-osseous metaplasia. Ulceration of atherosclerotic plaques with thrombus formation is the only lesion seen in man that has not been observed in cholesterol-fed chicks. (3) Cholesterol feeding apparently aggravates and intensifies spontaneous lesions. It would appear that the fibrotic spontaneous lesion is a site of predilection for cholesterol and lipid deposition, with subsequent evolutionary atherosclerotic changes. (4) Despite persistent dogma to the contrary, our experiments conclusively demonstrate that atherosclerotic lesions are reversible. (These findings are in accord with accumulated observations in man and rabbit.) Horlick and Katz demonstrated in the cholesterol-fed chick that in the weeks following cessation of cholesterol feeding, definite regression and
healing of cholesterol-induced lesions occurred. Previously existing moderately severe atheromatous lesions apparently completely healed and disappeared. More advanced lesions underwent partial resolution and evolution.

The question has been repeatedly posed: are experimental and human lesions morphologically similar? Even the most severe critics of the cholesterol concept of atherosclerosis cannot fail to note the conspicuous similarities between cholesterol-induced lesions—whether in rabbit, dog or chick—and the lesions seen in man. Taking into consideration the anatomic differences among the various species in architecture of the great vessels, the similarities between experimental cholesterol-induced and human lesions are indeed remarkable. That this resemblance is more than fortuitous is further emphasized by the fact that such lesions have not been produced experimentally by any other means.

**Amount and Duration of Cholesterol Feeding**

Our initial experiments on chick atherosclerosis placed us in a position to study possible exogenous and endogenous factors influencing the three types of avian lesions. However, preliminary to such investigations it became essential to quantitate the relation of the amount and duration of cholesterol feeding to the degree of hypercholesterolemia and to the incidence and severity of atherosclerosis. Toward this end several experiments were accomplished utilizing various levels of cholesterol in the diet and various feeding periods. Further, in order to permit quantitative evaluation of lesions, a system for gross grading of aortas for atherosclerosis was devised. This grading method, like others, is undoubtedly empiric and subjective. However, when employed with necessary precautions to guarantee treating all specimens as unknowns, it yields reliable information as to both incidence and severity of lesions. Hence it makes possible quantitation of pathologic data. By means of this grading method a general relationship is readily demonstrable between atherogenesis (incidence and severity of lesions) and cholesterol ingestion (amount and duration of sterol feeding). Thus with ranges of dietary cholesterol between 0.25 and 2.0 per cent (plus 5 per cent cottonseed oil), biochemical and pathologic alterations are quantitatively parallel. This is true both with chicks placed on experimental diets at 4 to 6 weeks of age and when 1 day old. In the course of these studies an additional basic methodologic principle became clear: In order to assess accurately the influence of various factors on experimental atherosclerosis, studies must be controlled with respect to each and every one of the following factors—age, sex, initial weight, feed intake, final weight and (at least in the chick) season. Attention to these elements in experiments on atherosclerosis insures a high degree of reliable quantitation in results.

**Undernutrition and Overnutrition**

Based on these studies it became possible to determine the influence of various factors on experimental atherosclerosis. The influence of one such factor, undernutrition, was explored in several experiments. In an early experiment, Dauber and Katz demonstrated that underfeeding per se (without cholesterol supplement in the diet) failed to induce lesions of the cholesterol type or to influence spontaneous atherogenesis. Subsequently, Rodbard and co-workers analyzed the effects of undernutrition on cholesterol-induced lesions. In one series of experiments chicks were fed mash containing either 0.25 per cent cholesterol plus 5 per cent oil or 2 per cent cholesterol. At each dietary level, one group of birds was permitted to eat ad libitum, another was given only 60 to 70 per cent as much mash. Chicks on this reduced dietary intake received less cholesterol than the controls given the same mash ad libitum. Nevertheless the semistarved birds tended to have a more severe hypercholesteremia than the controls. At the conclusion of the experiment incidence and severity of atherosclerosis in the undernourished cockerels were as great as or greater than in the control birds on an unrestricted diet of similar composition. Apparently the underfed birds were not readily able either to draw upon the elevated plasma lipids as a source of calories or to dispose of the exogenous cholesterol.
load. Thus it may be concluded that cholesterol remains an atherogenic stimulus regardless of the level of over-all caloric intake. It may be further suggested that in Europeans and Americans, where a definitive correlation has been shown between obesity and intensified atherogenesis, this influence of obesity may be attributable to the associated increased intake of specific atherogenic material, that is, cholesterol.

In another series of experiments, Rodbard and co-workers approached the same general problem somewhat differently; chicks were fed cholesterol diets during repeated short intervals. During interim periods (one to five days in duration), they were either starved or fed plain mash. A third group subsisted on the cholesterol diet continuously. The cholesterol-fed birds, starved during alternate periods, developed marked hypercholesteremia and atherosclerosis, resembling the findings in cockerels on a continuous cholesterol mash diet. In contrast, chicks alternating between cholesterol diet and regular mash exhibited only slight hypercholesteremia and atherosclerosis. Thus intermittent periods of starvation apparently hindered, rather than facilitated, the disposal of exogenous cholesterol load, whereas intermittent periods of regular mash facilitated such disposal. Over-all adequate intake of a balanced diet apparently made a key contribution to preventing dietary induced hypercholesteremia and atherosclerosis. Whether this effect is mediated by dietary or endocrine factors, or both, remains to be elucidated. These findings suggest the possibility that in man the effects of exogenous cholesterol on cholesterolemia and atherogenesis may be at least in part regulated by over-all nutritional status.

In contrast to these experimental studies on semistarvation, only limited observations are available on the effects of overfeeding on experimental atherogenesis. To our knowledge only one report in the literature deals with this problem. Wolfe and associates indicate that force fed geese exhibit an increased incidence of both spontaneous and cholesterol-induced atherosclerosis. Evaluation of these findings awaits publication of the complete data. Since obesity, the level of food intake in general, and cholesterol intake in particular have all been implicated as important factors in human atherogenesis, further experimental studies are indicated on excessive dietary intake and atherosclerosis.

Neutral Fat in Diet

Among other exogenous factors possibly influencing atherogenesis, neutral fat intake has been studied experimentally by us. In initial experiments, Dauber and Katz observed no effect of 20 per cent cottonseed oil diets on lipemia or spontaneous atherogenesis in chicks. More recently Stamler and co-workers extended these observations to chicks fed mash supplemented with 5 per cent cottonseed oil. Here again normal plasma lipid patterns were observed, no effect on spontaneous atherogenesis was noted, and no lesions of the cholesterol-induced variety supervened.

These experiments cannot be interpreted as indicating that neutral fat ingestion is not a factor in human atherogenesis. Clinically, neutral fat and cholesterol ingestion are almost invariably combined—in contrast to the foregoing experiments. Considerable evidence exists that under such circumstances triglyceride influences cholesterol metabolism. Moreover, a recent experiment in our laboratory also indicates that under certain circumstances neutral fat ingestion affects cholesterol metabolism and atherogenesis in the chick. Briefly, Stamler and associates demonstrated that defed pancreatized chicks respond differently to cholesterol feeding, depending on the presence or absence of cottonseed oil in the mash.

Cholesterol-Free and Fat-Poor Diet

In other studies on exogenous factors, Horlick and his colleagues analyzed the effects of a specially prepared defatted mash on both spontaneous and stilbestrol-induced atherosclerosis in cockerels. They also investigated the influence of such a low fat, cholesterol-free mash on regression of cholesterol-induced atherosclerosis occurring upon cessation of cholesterol feeding. In the first study, chicks receiving defatted mash developed spontaneous aorta lesions later than birds subsisting
on regular mash; these lesions were less extensive and severe. However, lesions were present. Hence the defatted mash diet failed to eliminate spontaneous arteriosclerosis in the chicks although it may have retarded this pathologic process. Apparently an exogenous source of lipid and cholesterol is not essential for spontaneous arteriosclerosis in the chick. If plasma lipids play a key role in such spontaneous arteriosclerosis, endogenous sources continue to make available sufficient lipids for this process. It might appear from these experiments that the spontaneous lesions produced are primarily arteriosclerotic and only secondarily atherosclerotic.

In a second experiment, Horlick and Katz observed that a defatted mash was without significant effect on the endogenous hyperlipemia and hypercholesteremia induced by stilbestrol implantation in cockerels. Stilbestrol-induced atherosclerosis developed concomitantly with the hypercholesteremia in these estrogen-treated birds fed a defatted mash.

In a third study with defatted mash, the influence of this diet on regression of cholesterol-induced lesions was analyzed by Horlick and Katz in chicks rendered arteriosclerotic by a prolonged period of cholesterol feeding. The specially prepared defatted mash was no more valuable in effecting the regression of atherosclerotic lesion than the regular chick starter mash.

The fact that spontaneous arteriosclerotic lesions occur with cholesterol-free, fat-poor diet, and that this diet had no obvious effect in eliminating the endogenously induced lesions casts some doubt on the view that low fat diets in man exert any marked influence on atherosclerosis. The conclusive proof for such a view must still be forthcoming. It is not at present available. It seems pertinent to suggest that in the present state of knowledge, use of low fat, low cholesterol diets in man would appear justified only (a) in cases with abnormally high cholesterol blood levels and (b) in patients with more than one episode of recent myocardial infarction, who have blood cholesterol levels at the upper limits of normal or above normal. Furthermore, the dietary restriction under such circumstances must be drastic; for example, a diet of 25 to 50 Gm. of fat per day must be rigidly adhered to, and it must be persisted in for long periods of time. The obvious difficulties of pursuing such a program and the inconclusiveness of the results obtained should make one hesitant to undertake widespread use of such dietary restraints until the controversy concerning the effects is settled conclusively one way or the other.

**Lipotropic Factors**

Among dietary factors possibly influencing human and experimental atherosclerosis, greatest attention has focused on lipotropic factors. Until recently their value was not clear, since contradictory findings were reported concerning the effects of these factors. This confusion has been eliminated in the last year or two. Experiments in chick, dog and rabbit yielding negative results are particularly decisive in indicating that at least choline is of no value in the prophylaxis or therapy of atherosclerosis. In this laboratory Stamler and co-workers studied the possible prophylactic influences of choline and inositol in spontaneous, cholesterol-induced and stilbestrol-induced atherosclerosis in cockerels. In the studies of lipotropic factors in cholesterol-induced atherosclerosis, the effects of 1 per cent choline plus 1 per cent inositol were determined in birds fed three different concentrations of dietary cholesterol, 0.25, 0.5 and 2 per cent. In studies continuing from 15 to 35 weeks all experimental regimens were well tolerated; normal feed intakes and rates of weight gain were recorded throughout. All cholesterol diets produced hypercholesteremia and hyperlipemia of varying degrees, hypercholesteremia being the principle alteration in the plasma lipid pattern. Phospholipids rose, but disproportionately less than cholesterol so the plasma total cholesterol/lipid phosphorus (C/P) ratio increased significantly. At all levels of cholesterol feeding 1 per cent choline plus 1 per cent inositol failed consistently to lower hypercholesteremia and hyperlipemia. The lipotropic factors were without effect in ameliorating the minimal hypercholesteremia consequent upon feeding 0.25 per cent cholesterol mash. The data indicate that during the initial weeks of the experiment
The addition of lipotropic factors to the diet actually aggravated hypercholesteremia. The exhibition of the phospholipid precursors choline and inositol failed significantly to elevate plasma phospholipid levels sufficiently or to correct the abnormally high plasma total cholesterol–lipid phosphorous ratios. Choline and inositol had only a limited incomplete lipotropic effect on cholesterol-induced hepatic lipidosis and completely failed to influence the lipidosis in other organs, including the aorta. Similarly the lipotropic factors failed to reduce the incidence or severity of cholesterol-induced lesions in either the thoracic or abdominal aorta.

In the companion experiments, Stamler and co-workers demonstrated that the lipotropic factors were also without significant effect on the hyperlipemia of stillbelostrol administration and on either stilbestrol-induced or spontaneous atherogenesis. Recent reports of carefully controlled studies by Firstbrook and Davidson and associates similarly indicate that choline is ineffective as a prophylactic or therapeutic agent against cholesterol-induced atherosclerosis in rabbits and dogs. Insofar as studies in man are concerned, adequately controlled clinical data are lacking; the efficacy of lipotropic factors in the prophylaxis or therapy of human atherosclerosis has not been demonstrated. Hence we deem it necessary to emphasize that neither clinical nor experimental foundations are present to justify the widespread prescription of costly preparations of lipotropic factors for human atherosclerosis. Such procedure merits condemnation on both scientific and socioeconomic grounds.

**Aluminum Hydroxide Gel**

Since cholesterol undergoes an enterohepatic circulation, the possibility presents itself that the intestinal disposal of cholesterol might be enhanced, plasma cholesterol levels thereby lowered, and atherogenesis retarded. Rodbard and his colleagues have been carrying out experiments on this problem in our laboratory. Utilizing a specially prepared aluminum hydroxide gel, it has been possible to decrease hypercholesteremia and atherogenesis in cholesterol-fed cockerels. This problem is under further study in our laboratory at the present time.

**Cholesterol–Phospholipid Ratio**

A number of recent investigators have brought forward evidence from studies on man suggesting that the plasma cholesterol–phospholipid (C/P) ratio may be a key factor in atherogenesis. Since cholesterol-induced atherosclerosis in rabbit, chick and dog is uniformly associated with a disturbed lipid pattern characterized by hypercholesteremia, altered cholesterol–phospholipid ratios and chylo microemia the question arises: is the hypercholesteremia per se or the altered cholesterol–phospholipid ratio the decisive factor in atherogenesis? Can atherogenesis occur without an elevated cholesterol–phospholipid ratio?

Several studies in our laboratory bear upon this problem of cholesterol–phospholipid ratios and atherogenesis. First, it is noteworthy that estrogen-treated chicks, unlike cholesterol-fed birds, exhibit a plasma lipid pattern characterized by hyperphospholipemia in excess of hypercholesteremia, with a resultant lowering of the plasma cholesterol–phospholipid ratio. Aortic atherosclerosis eventually supervenes in birds subjected to prolonged estrogen treatment. Under these experimental conditions a fall in the cholesterol–phospholipid ratio prevents neither plasma lacticence nor aortic atherosclerosis.

In another experiment Stamler and Katz attempted to correlate plasma biochemical data and postmortem pathologic findings in individual chicks fed 0.25 per cent cholesterol mash for 35 weeks, beginning at 5 weeks of age. Analysis of these data reveal a close correlation between the level of hypercholesteremia and aortic atherogenesis in individual birds. In contrast, there was no correlation between presence or absence of induced lesions in the aorta and degree of elevation of the plasma cholesterol–phospholipid ratio. Thus, this experiment demonstrated a lack of correlation between plasma cholesterol–phospholipid ratios and aortic atherogenesis, together with a positive correlation between plasma cholesterol levels and aortic lesions.

Although these experiments indicate that
cholesterol–phospholipid ratios are not decisive factors for aorta atherogenesis, recent studies by Pick and associates in our laboratory indicate that cholesterol–phospholipid ratios and coronary atherogenesis may be closely interrelated. Thus, estrogen exhibition to cholesterol-fed chicks results in suppression of coronary atherogenesis. Concomitantly, the ratios are depressed to or toward normal, despite persistent cholesterol-induced hypercholesterolemia.

This finding of prophylactic inhibition of coronary atherosclerosis by estrogens may be a significant lead concerning the mechanism of the well known sex differential in human susceptibility to coronary atherosclerosis. Further, since cholesterol-fed chicks are not protected against aorta atherosclerosis by estrogens, but are protected against coronary lesions, it would appear that atherogenesis proceeds according to different biologic laws in different vascular beds. Evidence is extant indicating a similar phenomenon in man. Hence, investigation of experimental atherosclerosis should not be confined to aorta lesions, lest significant findings in such important beds as the coronary and cerebral be entirely overlooked.

Thyroid

In addition to the foregoing studies, this department has carried on a number of experiments on the influences of various endogenous factors upon experimental atherogenesis. Among such factors studied to date, thyroid hormone has been found to be far and away the most effective agent influencing experimental atherogenesis. In an initial study in this laboratory, Dauber and associates demonstrated that desiccated thyroid significantly depressed the hypercholesteremia of birds given diets supplemented with 0.5 to 2.0 per cent cholesterol plus 20 per cent cottonseed oil. In accordance with these plasma lipid patterns thyroid hormone significantly decreased the incidence and severity of cholesterol-induced atherosclerosis. In a subsequent study, Stamler and co-workers extended these observations to stilbestrol-induced atherosclerosis in cockerels. Although desiccated thyroid only temporarily depressed the hyperlipemia of chronic stilbestrol administration, it was remarkably effective in reducing the incidence and severity of lesions in the aorta.

The mechanism or mechanisms of this effect of thyroid hormone on atherosclerosis remain obscure. Various hypotheses have been advanced by different investigators, among them that thyroid exerts its effect directly on cholesterol metabolism, reducing the hypercholesteremic stimulus to atherogenesis; that thyroid hormone affects the tissue accumulation of cholesterol; that thyroid hormone alters vascular permeability. Unfortunately, little is known concerning the mechanisms whereby thyroid affects lipid metabolism. Recent studies with radioactive tracers indicate that thyroid influences the hepatic degradation of the lipids, effecting lipid depletion.

In one of our studies Stamler and his colleagues undertook to determine whether the influence of thyroid hormone on lipids and atherosclerosis is a simple by-product of induced hypermetabolism. A comparison was made of the effect of desiccated thyroid and of the hypermetabolism-inducing drug dinitrophenol on plasma and tissue lipids and atherogenesis in cholesterol-fed chicks. Despite the similar hypermetabolism-inducing potential of thyroid and dinitrophenol, markedly different effects of the two substances were observed in these cholesterol-fed birds. Unlike the birds given thyroid hormone, the dinitrophenol-fed animals failed to exhibit a depression of either hypercholesteremia or atherogenesis. This experiment therefore suggests that the effects of thyroid hormone on lipid metabolism and atherogenesis cannot be attributed solely to any generalized nonspecific increase in energy exchange it induces. Rather than being nonspecific by-products of increased metabolic rate, these actions of thyroid on cholesterol metabolism would appear to be effected via specific metabolic reactions involving hormone and lipids—reactions whose pathways are today obscure.

Pancreas

In view of the increased incidence and severity of atherosclerosis in clinical diabetes, the role of the pancreas (as well as the other endocrine glands involved in the pathogenesis
of diabetes) in experimental atherosclerosis merits considerable attention. Until recently, experimental work on this aspect of atherosclerosis was very limited in scope. A few reports indicate that atherosclerotic lesions eventually develop in chronic diabetic dogs, particularly in completely depancreatized insulin-maintained animals subsisting on a diet of moderate to high fat content without supplementary raw pancreas or lipotropic factor. 

Experimental work on these problems in the rat, a species which to date has proved to be particularly resistant to cholesterol-induced atherosclerosis, has been even more limited. In this and other departments no success has been obtained in efforts to induce atherosclerosis by cholesterol feeding in alloxan diabetic rats. In rabbits, several workers report cataracts, but no atherosclerosis, following long-standing alloxan diabetes. When alloxan diabetes is combined with cholesterol feeding, the unexpected observation was made that atherogenesis tended to be retarded. Duff and Paine attributed this finding to the interrelations between cholesterol and phospholipid (normal C/P ratios) in such animals. It has also been suggested that this inhibitory phenomenon may be due to undernutrition and emaciation in alloxan diabetic rabbits.

It may be noted at this point that the study of the role of lipotropic factors in experimental atherosclerosis has its roots in the original observations that totally depancreatized, insulin-treated dogs can be chronically maintained only if fed a supplement of raw pancreas or its decisive lipotropic factors, lecithin or choline. Hence, in a certain sense the studies on lipotropic factors (which, as already noted, have yielded negative results) are an aspect of the problem of the role of the pancreas in experimental atherosclerosis.

In our laboratory, we have studied this over-all problem in chicks. This animal is readily amenable to total pancreatectomy or alloxanization. In an initial study, Stamler and co-workers determined the effect of pancreatectomy on plasma and tissue lipids and on the three types of chick atherosclerosis. In totally depancreatized cockerels fed a regular mash, free of cholesterol or other supplements, both plasma lipid and carbohydrate level are within normal limits. These birds exhibit no overt signs of metabolic disturbance. Lesions of the induced variety do not develop, although these chicks exhibit a tendency to intensified spontaneous arteriosclerosis. Stilbestrol-treated, depancreatized birds also exhibit a plasma lipid pattern essentially similar to that of their unoperated controls.

When a supplement of cholesterol and cottonseed oil is added to the mash of depancreatized cockerels, a plasma lipid pattern emerges that is significantly different quantitatively from that of their paired controls. They consistently exhibit a far more severe hypercholesteremia; this is accompanied by intensified atherogenesis. In contrast, when depancreatized chicks are fed a mash containing cholesterol, but devoid of a cottonseed oil supplement, they exhibit a plasma lipid pattern essentially similar to their unoperated paired controls on a similar diet. Thus, the addition of neutral fat to the diet apparently plays a decisive role in the development of an inordinate hypercholesteremia in pancreatectomized cholesterol-fed chicks. Since depancreatized cockerels fed a cholesterol–cottonseed oil mash exhibit a response different from their paired controls, it may be concluded that this dietary regimen has brought out subtle defects of lipid metabolism in these birds. Similarly, it is possible to bring out subtle defects in carbohydrate metabolism. Although chronically depancreatized chicks on regular diet exhibit gross derangement in neither plasma lipid nor glucose level, the administration of adrenocortical extract to such birds results in a marked hyperglycemia, far in excess of that seen in unoperated controls, and reaching levels well into the severe diabetic range.

These two experiments indicate that in the chick, as in man, the pancreas apparently plays an important role in the metabolism of both the lipids and carbohydrates. This biologic similarity emerges despite initial data indicating that pancreatic deficiency has different effects on metabolism in chick and man. Our findings suggest that these apparent differences between man and chick are not necessarily
qualitative ones, but rather at least in part quantitative. The relationships of both lipid and carbohydrate metabolic effects to atherosclerosis in the chick are under further investigation in this laboratory.

**Hypertension**

In view of the frequent clinical observation that hypertension is associated with intensified atherosclerosis in man, we undertook to study the interrelationship between these two pathologic processes in the experimental animal. The demonstration by Lenel and associates in our laboratory that salt feeding induces a significant chronic rise in blood pressure in chicks made it possible to carry on such experiments. In an initial study, Stamler and Katz compared atherosclerosis in salt hypertensive and normotensive cockerels subsisting on a diet of regular mash without a cholesterol supplement. The experimental regimens had no effect on plasma cholesterol concentration. Despite a significant elevation of blood pressure in the salt-treated animals, no gross lesions of the induced type were observed, nor was there any clear-cut intensification of spontaneous lesions. Thus, salt hypertension had little or no effect in grossly intensifying spontaneous arteriosclerosis in cockerels.

In a subsequent study, Stamler and co-workers analyzed the effect of another type of hypertension on atherosclerosis in chicks subsisting on plain mash; namely, desoxycorticosterone acetate (DCA)–salt hypertension. Here again, in the absence of a supplement of cholesterol in the diet, no lesions of the induced type were observed, nor was there intensification of spontaneous lesions. It would appear that in the presence of consistent normocholesteremia, hypertension is ineffective as an atherogenic stimulus, both with respect to causing the appearance of induced lesions and with respect to intensifying spontaneous lesions. This finding is in accord with observations in this and other laboratories with several other species, including rabbit, dog, sheep and goat.

In contrast to such negative results, desoxycorticosterone-salt hypertension did intensify cholesterol-induced atherosclerosis in cholesterol-fed cockerels. This observation, too, is consistent with the findings of other workers utilizing rabbits and dogs; namely, that hypertension intensifies atherosclerosis when accompanied by a cholesterol diet. The mechanism of this effect remains obscure. Regardless of mechanism, however, the facts available from these experiments support the view enunciated by several investigators: cholesterol is the decisive factor in the pathogenesis of atherosclerosis, whereas hypertension acts rather as an intensifying agent, exerting this effect when the lipid metabolic situation favors atherosclerosis.

**Vascular Damage**

Some workers attribute the effect of hypertension on atherosclerosis to a vascular damage presumably caused by hyperpiesis. Fundamental to such a concept is the problem: is pre-existent vascular damage a prerequisite for lipid deposition and atherosclerosis? Numerous attempts were made to clarify this problem experimentally. A host of noxious agents were tested for atherogenic effect, including bacteria, toxins, drugs, hormones, mechanical and thermal trauma. Although various lesions were experimentally produced by these methods, atherosclerosis was never produced. Thus it would appear that in the absence of an essential lipid metabolic alteration, vascular damage, per se, is ineffective as an atherogenic stimulus. On the other hand, experimental and clinical data from a number of sources indicate that foci of vascular damage are sites of predilection for deposition of atherogenic material, provided the necessary lipid metabolic alteration is present.

Some of the experiments in our laboratory bear upon this problem. In a recent study by Rodbard and his colleagues, 1 day old cockerels were placed on a diet of chick starter mash supplemented with 2 per cent cholesterol plus 5 per cent cottonseed oil. Weekly sacrifice of birds on this regimen revealed gross cholesterol-induced lesions of the aorta in birds as young as 5 weeks of age. It is apparent that these chicks had juvenile arterial tissue. Their vascular wall had not yet been subject to necrobiotic senescent alterations. More-
over, during their brief 5 weeks in the laboratory there was no indication in these healthy birds of the operation of pathologic stimuli noxious to the vessel wall other than the hyperlipemia itself. The occurrence of significant gross atherosclerosis in these cockerels suggests, therefore, that atherogenesis may proceed solely on the basis of deranged lipid metabolism, provided this is severe enough in degree and duration. On the other hand, Schlichter and associates have shown in dogs that vascular damage to the outer third or half of the aorta produced by cauterization focally alters the arterial wall, so that the subjacent intima becomes a site of predilection for deposition of administered atherogenic material (cholesterol). These and supplementary data of a similar nature from other laboratories lead us to agree with Autschakov's original concept, advanced many years ago, that cholesterol may be a primary atherogenic stimulus. We are also in accord with his further concept that vascular damage may play a role in the arterial deposition of lipids. These ideas, called the "combined theory" by Autschakov, recognize that a number of different factors are involved in the pathogenesis of atherosclerosis; they also recognize the key role of the lipids in atherogenesis, whereby a severe enough derangement of lipid metabolism may result in atherogenesis in previously normal vessels; they further recognize that with less marked derangements in cholesterol metabolism, atherogenesis may still proceed, particularly in sites of predilection produced by previous vascular damage.

Pituitary and Adrenals

In connection with these concepts of the role of vascular damage in the pathogenesis of atherosclerosis, considerable attention has recently been focused upon the influence of the adrenal steroids, particularly since several workers have demonstrated vascular injury in animals chronically treated with desoxycorticosterone acetate and other corticoids. This problem assumes further importance in view of the fact that clinical endocrine disorders characterized by excessive secretion of adrenal steroids (e.g., Cushing's syndrome) are associated with intensified atherogenesis. Introduction into clinical therapeutics of adrenocorticotropic hormone (ACTH) and cortisone further highlights the need to investigate the possibility that steroids may be atherogenic, particularly since chronic treatment with cortisone may result in a significant rise in the plasma cholesterol concentration of man. Finally we should take note of the fact that the adrenal cortex plays an active part in sterol metabolism as is indicated by their high concentration of cholesterol, their ability readily to discharge cholesterol, to synthesize cholesterol, and to accumulate ingested cholesterol. We have already briefly referred to the results of our studies on atherogenesis in desoxycorticosterone acetate-salt treated birds. Although moderate hyperpiesis supervened, no effect was observed on spontaneous lesions. However, cholesterol-induced atherogenesis tended to be moderately intensified. A more recent experiment by Stamler and associates further demonstrated that cortisone intensified aorta and coronary atherogenesis in cholesterol-fed chicks, although this steroid did not alter blood pressure or plasma lipid levels. Obviously these are not exhaustive experiments on the pituitary-adrenocortical axis and atherogenesis. The possible influences of other glyco- corticoids remain to be explored. Such studies are currently in progress in this laboratory. Also in need of further study are the complex interrelationships between the hypothalamic-pituitary-adrenocortical system and the other endocrines.

Aging

Among the endogenous factors possibly influencing atherogenesis, careful consideration must be given to the problem of age. A recent study by Rodbard and associates in our laboratory yielded significant findings concerning the influence of age on cholesteremia and atherogenesis. These workers recorded age-associated variations in level of cholesteremia in cockerels on a diet of constant cholesterol content. Thus, during the first seven weeks of life, birds on a diet containing 2 per cent cholesterol plus 5 per cent cottonseed oil exhibited a cholesteremia in the range of 200
to 500 mg. per 100 cc. At about the eighth week, this cholesteremia increased spontaneously to a level of 800 to 900 mg. per 100 cc., although no change was instituted in the experimental regimen. This new high level of cholesteremia tended to maintain itself during the next 12 weeks. At about the twentieth week of age, with the same diet continuing, there was a secondary fall of the plasma cholesterol level to about 300 to 500 mg. per 100 cc. In accord with these plasma lipid findings, atherogenesis during the first seven weeks of life was slight, whereas it proceeded rapidly after the eighth week. This experiment indicates that endogenous factors varying with age influence the response of chicks to a diet containing cholesterol. These endogenous factors, conditioned by and varying with age, influence both cholesterol metabolism and atherogenesis. Preliminary evidence suggests that hormonal factors may play a key role in this varying pattern of endogenous control of cholesterol metabolism.

Gonads

Among other endogenous factors possibly influencing atherogenesis, attention must be focused upon the role of the gonads, particularly in view of the conspicuous sex difference in the incidence of coronary disease in human beings. Experimental work on the gonads and atherogenesis has been limited. An extensive investigation of this problem is currently in progress in our laboratory. As already indicated, estrogens have been shown to inhibit coronary lesions in cholesterol-fed cockerels. A few reports are extant of experiments in the rabbit. These observations are patently incomplete and require supplementation by more extensive investigation.

Species Differences

Among the problems that emerge in the course of studying atherosclerosis experimentally, perhaps the most intriguing is the one of species differences in susceptibility to lesions. Man, the chick and the duck develop atherosclerosis "spontaneously." The dog and the rabbit rarely do. Experimentally it is easy to produce gross atherosclerosis in rabbits or chicks. It is more difficult in guinea pigs, hamsters and ducks. It is still more difficult in dogs. To date it has proved impossible consistently to produce gross atherosclerosis in rats. Why these species differences in atherogenesis? In our laboratory Schlichter has suggested that they may depend on species variability in the adequacy of the vasa vasorum of the large arteries. On the other hand, considerable evidence exists indicating that species differences in atherogenesis may depend on species variability in lipid metabolism. Studies by Horlick and co-workers in our department, utilizing the intravenous cholesterol tolerance technic, suggest that such variations exist. Thus the disappearance time of intravenously injected cholesterol is not the same in three species investigated to date. For proportional amounts of cholesterol the disappearance time in the rabbit is about 72 hours, in the chick 24 hours, in the rat 12 hours. Recent ultracentrifuge and isotope studies also demonstrate species differences in lipoprotein and cholesterol metabolism. Moreover there are well-known marked species differences in the cholesteremic response to orally ingested cholesterol. In general, ease of hypercholesteremia production and ease of atherosclerosis production parallel each other among the various species.

Finally, it is noteworthy that only in the rabbit and chick (and perhaps in man) can atherosclerosis be produced with but slight hypercholesteremia, resulting from chronic cholesterol ingestion by these experimental animals. Undoubtedly further research will clarify the reasons for the foregoing species differences in lipid metabolism and atherogenesis. Undoubtedly such advances will be fruitful contributions to the ultimate solution of the whole atherosclerosis problem.

Minimal Cholesteremia

In the foregoing sections, we have briefly reviewed the experimental work of our department on atherosclerosis. As indicated, in many of our experiments the dosage of choles-
atherol utilized resulted in marked hypercholesteremia and organ lipidosis, as well as atherosclerosis. The criticism has been frequently advanced that lesions occurring under such circumstances are fundamentally different from those seen in most human beings (excepting those with gross xanthomatosis), since atherosclerosis frequently occurs in people with presumably normal or near normal plasma and tissue cholesterol concentrations. In view of this frequently advanced criticism, Stamel and associates undertook two long-term experiments to explore this problem further, particularly in an effort to produce cholesterol-induced atherosclerosis in the chick with minimal concomitant hypercholesteremia and organ lipidosis. It was found that a diet of 0.25 per cent cholesterol mash induces a minimal hypercholesteremia and organ lipidosis in chicks. Maintaining chicks on this diet for periods beyond 15 to 20 weeks had a profound effect on atherogenesis, in the presence of minimal changes in plasma and tissue lipid concentrations. A high incidence was found of moderately severe gross atherosclerosis of the cholesterol-induced type in the thoracic aorta. These experiments demonstrated clearly that significant cholesterol-induced atherogenesis proceeds in chicks fed a dietary level of sterol inducing little or no hypercholesteremia and organ lipidosis. These findings in the chick are in accord with previous experimental work in the rabbit. When they are evaluated in relation to the latest data on man, these observations assume particular significance. Thus, today a mass of data exists demonstrating that a "xanthomatous tendency" (minimal hypercholesteremia) prevails in many people who are victimized by atherosclerosis. Moreover, considerable evidence is available indicating that cholesterol "input loads" (the quantity of ingested cholesterol per unit time that the body must absorb, transport, metabolize, turn over and excrete) influences atherogenesis in man. This conclusion is reinforced by the recent studies of Gofman indicating that diet may vary the concentration of various classes and species tentatively implicated in the pathogenesis of atherosclerosis.

**Conclusion**

In conclusion, we may reiterate that a basic proposition has been the foundation of the work of this department on experimental atherosclerosis. The essence of this proposition is that altered cholesterol metabolism plays a key role in human atherogenesis. The corollary of this proposition is that study of experimental cholesterol-induced atherosclerosis—of the exogenous and endogenous factors controlling it—would yield knowledge of fundamental significance for our understanding of human atherosclerosis.

For many years some investigators rejected these concepts because atherosclerosis had been produced experimentally only in rabbits. The consistent production of gross cholesterol-induced atherosclerosis in omnivorous chicks and dogs and other species now compels rejection of this criticism. For many years some investigators maintained that experimental and clinical atherosclerosis were fundamentally different, since hypercholesteremia was a prerequisite for the former, whereas the latter occurred in many normocholesteremic persons. The consistent production of gross cholesterol-induced atherosclerosis in chicks and rabbits with minimal hypercholesteremia, as well as the demonstration of a "xanthomatous tendency" in many persons with atherosclerosis, now compels rejection of this criticism. During the last decade, particularly, research in both clinical and experimental atherosclerosis has brought overwhelming support to the basic tenet of the cholesterol concept of atherosclerosis. What conclusion must be drawn from this? Certainly present knowledge does not permit us to state that the atherosclerosis problem is solved. Certainly much basic clinical and laboratory research on both exogenous and endogenous aspects of the role of cholesterol in atherosclerosis lies ahead of us before solution is reached. What present knowledge affords us is a fundamental approach for future research—the approach embodied in what
we have termed the cholesterol concept of atherogenesis. In this direction, we predict, lies the solution of the atherosclerosis problem, and with it the eventual control and elimination of this malevolent disease.


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BIBLIOGRAPHY
