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Role of estrogens in atherosclerosis

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THE ROLE OF ESTROGENS IN ATHEROSCLEROSIS

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I. INTRODUCTION

One of the challenging problems concerning atherosclerosis is the influence of sex upon its incidence, progress, and complications. The relative infrequency of coronary artery disease in women before menopause and the all too frequent manifestation of this disease in men even during the fourth decade of life has long been realized. (1,2) This remarkable sex difference suggests that since the level of circulating estrogenic hormones is the most obvious difference between premenopausal women and other adults that these substances might in some way exert an inhibitory effect on the disease. Although the influence of sex on this disease has long been suggested, it has not been until recently, when some of the chemical factors in the pathogenesis of atherosclerosis have become better established, has the role of estrogens, as one of the possible etiologic factors, been given more attention.

The effects of estrogens have been tested in both man and in the chick with the purpose of determining whether these specific chemical substances favor or inhibit atherogenesis. In the following discussion an attempt will be made to examine some of the scattered, incomplete, and often conflicting information concerning the possible influence of estrogens in this process. Because of the many widely divergent

concepts, a brief review on the subject of atherosclerosis in general will also be considered. Attention will be focused on the possibility that there is something in the chemical constitution of the male that makes him more vulnerable.

Several basic questions will be stated here and the discussion will be concerned primarily in an attempt to answer them.

1. Is atherosclerosis an inevitable and an irriversible disease?

2. Does the lipid composition of the plasma in man differ from that of mammals which are not susceptible to spontaneous atherosclerosis?

3. Are variations in human predisposition to atherosclerosis signalized by differences in the lipid composition of plasma?

4. Is it possible that presumably undesirable characteristics of the chemical composition of human plasma could be modified by the administration of estrogens?

II. REVIEW OF CURRENT CONCEPTS ON ATHEROSCLEROSIS

A. Definition.

Atherosclerosis is a form of arteriosclerosis. Much confusion has come to exist because it is often used synonomously with "arteriosclerosis". According to Bell (3):

"All forms of arterial disease except those which are frankly inflammatory in nature are commonly called arteriosclerosis. Types of arteriosclerosis include: (1) Regenerative Intimal Thickening, (2) Elastic Intimal Thickening, (3) Ectasia, (4) Medial Calcification (Monckeberg's Sclerosis), (5) Arteriosclerosis, (6) Medial Fibrosis, (7) Intimal Atherosclerosis." It is only the latter which we will be considering in this paper. It is by far the most frequent form of arterial disease and the most devastating, since it is the chief cause of coronary artery disease and cerebral vascular accidents. It is called "intimal" because it begins in the intima and is usually restricted to this layer, but in advanced stages, especially in the smaller arteries, it penetrates deeply into the media.

The term atherosclerosis is derived from the Greek words athērē- meaning mush or porridge and -sklērosis meaning a hardening. Thus atherosclerosis might be defined as: the fatty degeneration and/or infiltration of lipids of the walls of the arteries within and beneath the intima with resultant formation of plaque-shaped lesions.

B. History.

Atherosclerosis is by no means a new disease for Ruffer finds that old Egyptian mummies suffered from arterial lesions identical with those prevalent today. (4) Yet 3,000

years later we must admit that our knowledge concerning this all important disease is seemingly only beginning to take order. One might wonder why man has waited so unreasonably long before undertaking to do something about a disease so prevalent and so lethal. Perhaps since the disease has long been considered "an inevitable process of aging" and an entirely "irreversible" process has there been such a stagnating atmosphere of helplessness and hopelessness and thus hindering research on this disease. Why attempt to cure the "inevitable" has been the common attitude! Even today this sense of defeatism and futility in the minds of many remains as a handicap to further progress. Indisputable post-mortem evidence has shown that clinically significant, marked atherosclerosis undoubtedly occurs in some very young people and is minimal in some very old people. These findings indicate quite clearly that it is not an inevitable by-product of senescence. (5,6) Thus senescence and atherogenesis are two distinct and not necessarily interrelated processes. That artherosclerosis is absolutely non-reversible has also been refuted by considerable experimental and clinical evidence. Pick and associates have demonstrated regression of previously induced coronary atherosclerosis in cholesterol-fed cockerels as will be discussed more fully later. (7) Wilens has also found

that resorption of arterial atheromatous deposits occurs in wasting disease. (8)

Because of the advances in the study of cholesterol-lipid-lipoprotein metabolism which have been made possible by the discovery of the ultra-centrifuge and other advances in chemistry there has been much progress in the study of this disease during the recent decades. Yet, with new discoveries great caution must be taken or hasty, premature conclusions will result. In this regard Page (4) has made a very appropriate comment. "We must expect atherosclerosis to be cured and its cause proclaimed at least twice a year for the next ten years or so. It is part of the natural history of discovery that emotions outpace logic. This is a healthy sign though, for it must be remembered that this is a new field with relatively poor backlog of knowledge and everything that turns up is new; even the investigators are new and quite shiney."

C. Incidence.

1. Overall.

Man is the only animal in which atherosclerosis develops naturally. Dogs, cats, rabbits, rats, guinea pigs, and higher arthropods seldom, if ever, develop spontaneous atherosclerosis. (9) Statistics indicate that half of those who die at the age of fifty and more than 85 per cent of those who die after the age of seventy-five are afflicted with a moderate or advanced

degree of atherosclerosis. According to a report by the National Heart Institute, atherosclerosis is the leading cause of death in the United States, claiming at least 200,000 victims or a minimum of one-seventh of those dying from all causes annually. It has been shown that about 90 per cent of all victims of coronary artery disease succumb to coronary atherosclerosis. (5,10) This entity alone takes a toll of lives each year nearly as large as do all malignant tumors. These estimates are surely very conservative for almost certainly many deaths which are attributed to other causes are actually due to atherosclerosis or its complications, i.e., Diabetes Mellitus.

2. Sex.

The higher incidence of atherosclerosis in men is well recognized. Hedly has noted that under the age of forty, predominance of the incidence of coronary artery disease in males is 25:1, and in the decade from forty to fifty a ratio of 5:1, then tending to become progressively equal thereafter. (11) The most decisive evidence of a greater incidence of coronary artery disease in men is afforded by the observations of Schlesinger and Zoll. (12), the results of whose injections and dissections of coronary arteries are present in Table I.

Table I. Incidence of Coronary Artery Occlusion In Men And Women At Varying Ages. (Schlesinger and Zoll)

Age (yr.)	Sex	Total Number of Hearts	Hearts with Occlusions	
			Number	Per Cent
20-39	M	44	1	2.3
	F	19	1	5.3
40-59	M	85	20	23.5
	F	56	2	3.6
60-79	M	115	43	37.4
	F	65	21	32.3
80 on	M	9	4	44.4
	F	7	2	28.6
Total		400	94	23.5

It can be seen here that in every age group occlusions were most frequent in men.

To further emphasize this sex difference as to incidence, White, Ackerman, and Dry (13) determined the degree of coronary atherosclerosis in two series of autopsy cases of 600 hearts of men and women, 100 from each decade from ages thirty to eighty-nine. Their study revealed the degree of narrowing of the coronary arteries in general was more severe in men in the earlier decades, increasing in intensity with age and becoming maximum during the fifth to sixth decade. In women, the degree was minimal up to the age of forty and then tended to rise steadily, but not reaching the intensity that men exhibited until between the seventh to eighth decade.

They found that women in the sixth decade exhibit about the same degree as men in the fourth; in the seventh decade their involvement corresponded to that of men in the fifth. (14)

From these several investigations, it would seem quite apparent that atherosclerosis, particularily involving the coronary arteries, occurs at an earlier age in men. The relative immunity of women to its development, especially in the premenopausal age group, is obviously also quite well shown. The possible role of estrogens as affording the latter some degree of protection has often been speculated, and it will be shown later that recent discoveries have proven significant differences in the lipid patterns, which show the lipid composition in the plasma of young women is such as might be expected to afford some degree of protection.

3. Racial Differences.

Considerable data has accumulated indicating that differences exist among races as to the incidence and severity of this disease. (6,13) In general, it has been shown that Italians, Spanish, Costa Ricans, Guatemalan Indians, Okinawans, Chinese, Japanese, Ceylonese, and Bantu natives, who subsist on diets low in cholesterol and lipids are remarkably free of atherosclerotic disease. Whereas, the inhabitants of the United Sates, and particularily amongst the Jewish race, the disease is all too prevalent.

4. Predisposing Diseases.

Certain diseased states including diabetes mellitus,

hypothyroidism, biliary obstruction, the nephrotic syndrome, and essential (familial) xanthamatosis are all associated with premature, severe atherosclerosis. It has been suggested that there probably is a familial or hereditary factor involved in these specific diseases and perhaps in families where several fatalities from the complications of atherosclerosis have occurred. It is interesting that the sex difference in the incidence of coronary artery disease which is so evident in the general population is entirely obliterated in the presence of these strongly atherogenic diseases. Bell and Clawson (15) were able to show that in the presence of long standing diabetes in the period before the age of forty the incidence of fatal coronary artery occlusion was the same in females as in males. The reason for this apparent lack of protection remains obscure.

D. Pathogenesis.

Recognition of the multiplicity of factors influencing atherogenesis serves to highlight the complexity of the pathogenesis of this disease. However, this in no way negates the basic conclusion--arrived at on the basis of an overwhelming mass of factual data--that the key factor in the pathogenesis of atherosclerosis is altered cholesterol-lipid-lipoprotein metabolism. This fundamental concept serves as the foundation and point of departure of the recent research assault upon atherosclerosis--an assault which is greatly enriching the understanding of this disease and is

pointing to an ultimate solution of this problem. (16) However, there is clear evidence that anatomical factors, including sub-endothelial fibroblastic proliferation, defects in the inner elastic lamella, and changes in the ground substance are also quite important and may even proceed the lipid changes. (4)

Page (4) has presented an excellent outline of the probable factors involved in atherogenesis and will be given here:

1. The anatomy, biochemistry, and physiology of the vessel wall, all of which are hereditarily conditioned.

2. The lateral arterial pressure and rate of filtration.

3. The composition of the plasma.

4. The responsiveness of intimal tissues to filtered products and their degradation products, normal and abnormal.

5. The metabolic capacity of the vessel wall.

6. Changes in filtration capacity of the vessel wall, such as may result from age, hypertensive and infectious diseases, and metabolic disorders.

With these factors in mind the "filtration" concept has been proposed. It is based on the view that atherogenesis is due to tissue reaction to substances filtered from the plasma as lipoprotein by lateral arterial pressure and deposited in the intima as "foreign" lipid. Most of the filtered materials pass on harmlessly to be picked up by the adventitial capillaries or the lymph. But some stay behind, either because the vessels fail to function properly

as a filter or because the size, shape, and charge of lipoproteins is such as to allow them to stick. Changes in the arrangement, amount, and chemical nature of subendothelial ground substance conceivably may initiate a focal change in filter function. The nature of the reaction which occurs depends on the nature of the lipid deposited and the responsiveness of the tissue to it. Perhaps the important thing which needs emphasis to support this concept is the basic similarity in the initial relative composition of the vessel wall and the plasma lipids. This supports the view that the source of the vessel wall lipid is plasma lipid.

2. Alterations of cholesterol-lipid-lipoprotein metabolism.

In general, there are four main factors in the plasma lipid composition which are felt to be of significance in atherogenesis: (1) Cholesterol (free and esters), (2) Cholesterol/Phospholipid ratio, (3) Lipoproteins, and (4) Sf 10-20 (Gofman) bodies.

In the atherosclerotic patient, there is generally an elevation of serum total cholesterol, but this may vary in amount and may be intermittent . Perhaps more important than the cholesterol level in atherogenesis is the cholesterol/phospholipid ratio. In atherosclerosis this generally has been found to be increased.

It has been known for some time that cholesterol and phospholipids do not circulate in the plasma in molecular solution,

but on the contrary, are combined with each other and with proteins as compounds or aggregates which are known as lipoproteins. (17) From the combined experience of many investigators it now appears that in normal human plasma and in plasma of many pathological conditions that essentially all of the cholesterol and phospholipid of plasma or serum are combined with one or two groups of proteins; with $alpha_1$ globulins as alpha lipoproteins, and with $beta_1$ globulins as beta lipoproteins. Perhaps the most significant difference from the standpoint of subsequent discussion is the cholesterol/phospholipid ratio of the two groups. The alpha lipoproteins are found to have cholesterol/phospholipid ratios by weight of about 0.50 while the beta lipoprotein as indicated by samples purified from pooled presumably normal plasma, have a ratio by weight of over 1.00. (18) This would indicate that the beta lipoproteins contain a considerable higher content of lipid substances. Blodgett has found that 25 per cent of blood cholesterol is combined with alpha lipoprotein and 75 per cent with the beta lipoprotein fraction. (19) In atherosclerosis the beta lipoprotein fraction tends to be increased at the expense of the alpha fraction. The Sf 10-20 (Gofman) bodies, which were the focus of the ultracentrafugal studies of Gofman and his associates, have not been precisely identified. The question whether they form a small part of the total beta lipoproteins is yet unsettled. They also tend to be elevated in atherosclerosis and certain investigators feel that they are of

crucial significance in its development. (20) Although the general trend of most of the investigational work at present tends to place the greatest pathological significance on the distribution of lipids between the alpha and beta lipoproteins, it would seem highly possible that all of the above factors are in some way involved.

3. Comparison of Plasma Lipid Values in Man and Other Mammals.

Since man apparently is the only mammal that develops atherosclerosis spontaneously, one might ask if there are any significant variations between his blood lipid pattern and that of other mammals, and if so in what ways do they differ? Although all of the criteria of this discussion have not been undertaken in any single laboratory, data from several sources permit a partial comparison with the dog and rabbit. The available information has been assembled in Table II. (17)

Table II. Comparison of Lipid Values in Man and Other Mammals, Data of Dogs, Rabbits, and Rats are from Unpublished Observations of Dr. Forrest E. Kendall.

	Man	Dog	Rabbit	Rat
Total cholesterol mg. %	200	210	51	67
Phospholipid mg. %	250	430	88	101
Chol./PL. ratio				
Plasma	0.80	0.49	0.58	0.67
Alpha lipoprotein	0.50	0.48	0.37	
Beta lipoprotein	1.25	0.51	0.76	
% total cholesterol in alpha lipoprotein	30	83	53	-
% total cholesterol in beta lipoprotein	70	17	47	-
S_f 10-20 mg. %	12	None	None	None

It will be seen that the averages for total cholesterol are almost identical in man and in the dog. In passing, it is worthy of note that if the concentration of cholesterol were to be regarded as the only criterion, no difference in predisposition to atherosclerosis would be anticipated. The phospholipid values on the contrary are much higher in the dog than in man, so consequently, the cholesterol-phospholipid ratio of the plasma of the dog is much lower. Although levels of alpha lipoprotein in man and dog are approximately equal, it can be seen that there is considerable difference in the beta lipoprotein levels. Similar differences are noted in the other mammals also. Thus it can be seen that there are many striking differences between the lipid composition of the plasma in these mammals which appear to be immune to spontaneous atherosclerosis and that in man who is so seriously predisposed.

4. The Effect of Age, Sex, and Diseases Predisposing to Atherosclerosis on the Lipid Composition and Distribution in Human Plasma.

Since all humans do not have the same degree of predisposition to the development of atherosclerosis, it is pertinent to inquire whether the variations in susceptibility are signalized by differences in the lipid composition of the plasma. A few of the better known clinical peculiarities may be mentioned briefly. No human is born with it. The disease is rare or absent in normal childhood. While it is not absent in all young women, its' complications are seldom seen before menopause. Both in its' extent and

in the frequency of its' complications, the disease becomes more threatening with age. Older men and women appear to be about equally affected. In certain diseases, and particularly in diabetes mellitus, the nephrotic syndrome and familial xanthomatosis, the disease develops early in life and tends to be unusually extensive. The effects of age, sex, and certain atherogenic diseases on the lipid composition and distribution of human plasma have been summarized in Tables III, IV, and V respectively. (17)

Table III. Effect of Age on the Lipid Composition and Distribution of Human Plasma.

	Fetus Cord Blood at Term	Normal Young Men and Women Age 18-35	Normal Older Men and Women Age 45-65
Total cholesterol mg. %	65	190	245
Phospholipid mg. %	123	223	229
Chol./PL. ratio			
Plasma	0.56	0.90	0.98
Alpha lipoprotein	0.43	0.53	0.50
Beta lipoprotein	0.82	1.32	1.36
% total cholesterol in alpha lipoprotein	43.0	29.3	23.1
% total cholesterol in beta lipoprotein	57.0	67.5	75.2
Sf 10-20 mg. %	0	10	15

Table IV. Effect of Sex on the Lipid Composition and Distribution of Human Plasma.

	Normal Women Aged 18–35	Normal Men Aged 18–35	Normal Women Aged 45–65	Normal Men Aged 45–65
Total cholesterol mg. %	187	197	252	239
Phospholipid mg. %	228	195	278	265
Chol./PL. ratio	0.87	0.97	1.00	0.95
% total cholesterol in alpha lipoprotein	34.3	25.2	23.4	22.9
% total cholesterol in beta lipoprotein	61.8	72.0	75.0	75.3
Sf 10–20	5	12	15	15

Table V. Effect of Diseases Predisposing to Atherosclerosis on the Lipid Composition and Distribution of Human Plasma.

	Normal Young Men and Women	Survivors of Myocardial Infarction	Nephrotics	Diabetics	Familial Xanthomatosis
T. cholesterol (mg. %)	190	259	577	254	423
Chol./PL. ratio	0.90	1.02	1.28	1.08	1.12
% T. cholesterol in a. lipoprotein	29.3	13.6	5.1	19.1	5.9
% T. cholesterol in b. lipoprotein	67.5	83.9	93.8	78.7	93.1
Sf 10–20	10	33	ea 200	ea 30	ea 250

It will be seen that young men and young women have about equal concentrations of cholesterol, but that in young women the concentration of phospholipid is higher, the cholesterol-phospholipid ratios tend to be lower and that there is a greater percentage of total cholesterol in alpha lipoprotein and a lower Sf 10–20 concentration. In contrast to this, it is remarkable that the lipid constitution

of plasma in men and women past the age of forty-five differs but little.

It would seem then, from the standpoint of analyzable chemical factors, that the lipid composition of the plasma of the newborn baby closely simulates or resembles that of the rabbit and rat and that sometime during childhood, and perhaps quite early, these characteristics are lost. Among adults, the young healthy woman deviates least from the pattern of newborn babies and immune mammals. It can also be seen that in diseases which are known to predispose strongly to premature atherosclerosis and in survivors of myocardial infarction that the pattern of lipid concentration and distribution deviates most widely from those of immune mammals and newborn human infants. They vary significantly from the patterns of the relatively immune young women as well.

Thus with increasing evidence that there is a correlation between atherogenicity and the variations in the chemical composition of the plasma, it is also becoming more apparent that the pattern of lipids in the plasma of young women is such as might be expected to afford some degree of protection. This data, however, so far can only be considered as circumstantial evidence, and cannot be used as proof that the relative freedom of young women from atherosclerosis is dependent upon the chemical constitution of the blood.

5. Hypothesis of mechanisms of action on how estrogens may inhibit atherogenesis.

Because the level of circulating estrogens is the most obvious difference between premenopausal women and other adults, it has been postulated that estrogens may in some way be involved in inhibiting atherogenesis. Oliver (21) has observed cyclic depression of the total cholesterol and cholesterol-phospholipid ratio in normal young women at the time of ovulation and has suggested that this might be related to maximum estrogen secretions at that time. Wuest, Dry, and Edwards (22) have found a more marked degree of coronary atherosclerosis in the hearts of bilateral oophorectomized women at autopsy than was noted in a control group of women of corresponding ages. This again suggests that estrogens exhibit some prophyllactic effect. The precise mechanism of how remains obscure. Broakedt (23) has proposed that since the alpha lipoprotein level has been shown to be consistently higher in premenopausal women and that the plasma proteins are both synthesized and degraded by the liver the ultimate control of lipid levels is probably mediated through this organ. He further adds that estrogens, per se, may have no direct effect on plasma lipids or the liver, but that their action may be achieved through other organs such as the adrenal cortex. Taurog (24), by the use of radioactive phosphorus, has shown that the livers in birds receiving diethylstilbesterol form a significantly increased amount of phospholipds. This again suggests that the liver and estrogenic substances are

in some way directly or indirectly involved in increasing the level of the serum lipid phosphorus. Damage to the liver by carbon tetrachloride has been shown by Wakerlin (25) to enhance experimentally-produced atherosclerosis in animals. From this brief discussion, it can be seen that if estrogens do cause an inhibitory influence on atherogenesis, just how they do this is still unknown.

6. The role of diet.

With all the discussion of atherogenesis centered about alterations in cholesterol-lipid-lipoprotein metabolism, one might reasonably conclude that dietary control of cholesterol and lipid intake might play a very significant role. Also, as noted previously, certain ethnic groups who subsist on diets low in cholesterol and lipids exhibit plasma lipid levels consistently lower, and are more often free of atherosclerotic disease. These facts do not justify any oversimplified idea that atherosclerosis is merely a problem of diet, pure and simple. Moreover, in defiance of such an explanation of atherogenesis, the fact remains that women are remarkably immune to coronary atherosclerosis, in the premenopausal decades--a phenomenon which can hardly be attributed to dietary differences. (16)

What then is the role of diet? Katz, Stamler, and Pick (16) feel that the ingestion over the years of a diet rich in cholesterol and lipid apparently is a prerequisite for the development of significant atherosclerosis in a population. Such a diet is apparently

an essential "trigger" for the atherogenic process. Once the trigger is pulled, individual differences--endogenous factors (determined hereditarily and otherwise)--come into play. The interrelationship is undoubtedly more complex, in that the nature of the given organism not only influences the response to diet, but the diet in turn influences the organism and its' endogenous response to diet over the years.

In general, diets low in cholesterol content are no longer being advocated. Since the body can synthesize about 2 grams of cholesterol daily as compared with the usual intake being only 0.2 to 0.4 grams in the average diet, it can be seen that the endogenous source is the most important. Because the body is able to synthesize cholesterol from acetate, a low fat, low caloric diet may be of more value. However, because a diet extremely low in fat content is not very pallatable and may result in deficiencies in essential fatty acids, it is recommended that the fat intake should not be less than 15 per cent of the total caloric intake. (4)

E. Clinical Manifestations and Diagnosis.

The difficulty of recognizing atherosclerosis clinically is notorious. Page states the problem very bluntly: "The answer to the problem of recognizing latent atherosclerosis is simple--we have no way." (4) Roentgenological surveys of the arterial system for abnormal patches of calcification, electro-

cardiographic, ballistocardiographic observations, exercise tolerance tests and even biochemical examination of the serum lipid composition may be helpful in indicating its' presence, but at best give only circumstantial evidence. No one of these is pathognomonic, and all of them fail to establish proof of the extent or even the presence of the process. Indeed, it is quite impossible at present to distinguish between the apparently normal adult and the very atheromatous individual who has not yet recognizable complications of the atherosclerotic lesion. Those who have exhibited myocardial infarction, angina pectoris, intermittent claudications may have suffered their disabilities because of strategically located patches of atheroma. In general, their arteries may be freer of atherosclerotic disease than those who have exhibited no symptoms or signs of illness.

F. Treatment.

Again as stated by Page (4), "properly speaking, there is no treatment." This perhaps seems rather negativistic, but when the cause has not been definitely established, and there are no methods of diagnosis, this would seem a more scientific attitude to take.

There are certain methods of treatment, none of which have been definitely proven to be of value, which have some suggestive evidence of their rational. These include: (1) Low caloric, low fat diet, (2) Iodides and thyroid, (3) Heparin-like substances,

(4) Sitosterol and other cholesterol analogs, (5) Estrogens. It is only the latter that we will be concerned with in this paper. Results of some of the animal and clinical investigational work will be taken up subsequently.

IV. THE EFFECTS OF ESTROGENS ON ATHEROSCLEROSIS IN THE DOMESTIC FOWL.

Since mammals other than man seldom develop spontaneous atherosclerosis and since many birds have a high incidence (26,27), the domestic fowl has been selected as a convenient experimental subject.

The spontaneous lesions of the arteries have been examined carefully by Dauber (28) and by Chaikoff and others. (29) Early in the life of the rooster and in the immature female, elevated, smooth longitudinal ridge-like thickenings develop in the abdominal aorta. Such lesions have not been observed in the ascending aorta and arch or in the coronary arteries. In cockerels, commercially fattened for market, lipid and cholesterol could be demonstrated in the lesions of the abdominal aorta. In cockerels reared in the laboratory throughout life on a mash devoid of cholesterol supplement, the thickened ridges of the abdominal aorta contained no demonstrable lipid. (30)

In the female birds at puberty and during the egg laying period there are much more extensive arterial changes. Nodular, ridge-like plaques in the descending thoracic and abdominal aortas are infiltrated with lipids which may be demonstrable by gross as

well as by microscopic examination. The ascending aorta and the aortic arch may be streaked with deposits of yellow fat. The involved area is smooth, and no fibrosis or true atheroma is evident. The change is limited to diffuse accumulation of lipid in the intima and media. Neither lipid-bearing foam cells nor cholesterol crystal clefts are readily demonstrable. No coronary lesions which are recognizably atherosclerotic can be shown in the spontaneous atherosclerosis of the domestic fowl either in the rooster or in the laying hen. Again the question must be asked whether this spontaneous deposit of fat in the laying hen is closely analagous to human atherosclerosis? (31)

In 1940, Enteman, Lorenz, and Chaikoff (32) reported the effects of crystalline estrogens on the blood lipids of birds. Anatomically and chemically, the changes produced by the estrogens both in cockerels and in immature females resemble the alterations exhibited by the pullet at puberty. The concentrations of cholesterol, phospholipid, and neutral fat greatly increased. The atherosclerotic changes in the vessels corresponded quite precisely to those previously observed at puberty. The arterial changes which result in cockerels and in immature female chicks following the administration of estrogens may mimic precisely those shown in the untreated mature laying hen. (29,33)

Extensive observations have been made on the arterial and chemical changes that follow experimental feeding of cholesterol

and excess of fat to chickens at varying ages. The lesions produced in this manner differ in many respects from those developed spontaneously or following the use of estrogen. From the beginning, they resemble human atheroma and include the formation of foam-cell plaques, necrosis, fibrosis, hyalinization, calcification, and cartilaginous and osseous metaplasia. Ulceration of the atherosclerotic plaques with thrombus formation is the only lesion of man which has not been noted in the chick. Lesions may form in the entire length of the aorta and in the coronary arteries. Accompanying these anatomic changes there is a great increase in the concentration of cholesterol, a disproportionately small rise in the phospholipids, and consequently, an elevated cholesterol-phospholipid ratio.

The observations of Katz and his associates deserve detailed attention for their work on the influence of estrogens in experimental atherosclerosis in domestic fowl. Some of their experimental work will be briefly reviewed here with comments and conclusions which seem appropriate also being included.

The first experiment shows age-conditioned spontaneous regression of atherosclerosis in cholesterol-fed chicks. In this experiment, 130 chicks were placed on a diet supplemented with 2 per cent cholesterol during the entire period of observation. It was noted at puberty (eight weeks) that diffuse lipid infiltration and atherogenesis was proceeding rapidly throughout the body. By fifteen weeks, distinct atherosclerotic plaques were observed. With sexual

maturation (20 weeks), it was noted that spontaneous regression of the atherosclerotic lesions began, despite continued cholesterol feeding. Animals placed on cholesterol diets at this time showed retarded development of lesions. From the twenty-six to seventy-four weeks, further regression was noted, but pin-point sclerosis on a thickened intima with occasional calcification remained. This experiment illustrates that the susceptibility to atherosclerosis in chickens depends to a large extent on endogenous factors dependent on the age period of the animals. Since regression of the lesions began with sex maturation of the chickens, the effect of estrogens is suggested as the mechanism for this action. (34)

A second experiment revealed actual inhibition of coronary atherosclerosis in cholesterol-fed cockerels by the administration of estrogens parenterally. It was found that the protection of the coronary vessels by estrogen was associated with changes in the total cholesterol-lipid phosphorus ratio. It was noted that estrogen failed to exert any prophyllactic effect against atherosclerosis of the aorta, however. It becomes apparent from this observation that atherogenesis does not proceed according to the same biologic laws in different vascular beds. This difference of susceptibility of various vascular beds has also been noted in man. (35)

In another experiment, this same group (36) has shown that estrogen reverses coronary atherosclerosis in cockerels which had

been previously induced by cholesterol feeding, despite continued feeding of the cholesterol diet. This estrogen-induced regression occurs in the presence of continued marked hypercholesterolemia, reversal of previously elevated total cholesterol-lipid phosphorus ratios to normal levels, and of persistent aorta atherogenesis. Although the mechanism of estrogen-induced regression of coronary lesions remains obscure, the authors have suggested several possibilities: (1) Estrogen may induce lipophage activity which effects movements of lipid towards the adventitia and perivascular tissue. (2) Likewise, estrogen may decrease the permeability of the endothelium for lipids. (3) The hormone may have an effect on the plasma lipid-lipoprotein complex. These possible mechanisms, obviously hypothetical at the present stage, can hardly account for the regression of coronary lesions without effect on the aortic atherogenesis. This finding may be related to metabolic differences (both quantitative or qualitative) between these vascular beds.

In view of the well known sex differences in human susceptibility to coronary atherosclerosis prior to age fifty, experiments were undertaken by this same group on the effects of gonadectomy on cholesterol-induced atherosclerosis in young growing chicks. Both male and female chicks thirteen weeks olds were maintained on a diet containing 1 per cent cholesterol plus 5 per cent cottonseed oil. It is interesting that the degree of hypercholesterolemia was comparable in all groups, male and female, castrate and intact,

hormone-treated and non-treated. Correspondingly, the incidence and degree of aorta atherogenesis was similar in all groups. Intact untreated females exhibited a low incidence of cholesterol-induced coronary atherogenesis (40 per cent), compared with gonadectomized untreated females (100 per cent) and intact untreated males (100 per cent). All groups receiving estrogen, males and females, intact and gonadectomized, were uniformly free of coronary atherosclerotic lesions. (37)

One of the most recent reports by this group was on their investigation to determine if inhibition of cholesterol-induced atherosclerosis in egg-producing hens occurs. (38) In this experiment, studies were conducted on oviduct-ligated as well as intact females to rule out any possible greater resistence to coronary atherogenesis being attributed to disposal of cholesterol from the body via the egg-laying process. The results in both groups showed resistence to coronary atherogenesis, blood lipid alterations and susceptibility of aorta atherogenesis which were identical to those produced by administering exogenous estrogens either orally or parenterally to cholesterol-fed cockerels, roosters, and immature chicks.

It is noteworthy from this that mature, gonadically active chickens and humans both exhibit a marked sex differential--in favor of the female of the species--in susceptibility to coronary atherosclerosis. This experiment, and also the proceeding ones,

lend further support to previous studies that estrogens play a key role in protecting the premenopausal women against coronary atherosclerosis.

IV. THE EFFECTS OF ESTROGENS ON ATHEROSCLEROSIS IN MAN

From the bulk of investigational work that has been done on the pathogenesis of atherosclerosis, it can be discerned that alterations in the composition and distribution of the serum lipids is the key factor. If these abnormalities are considered to be significant, it would appear that the primary goal of any therapeutic procedure should be directed towards altering these abnormal lipid patterns in the direction of those found in the premenopausal female and animals who appear to be immune to atherogenesis.

Because young women have less tendency to develop complications of atherosclerosis than young men, and because this apparent immunity is no longer apparent after menopause as has been shown earlier, the importance of the action of sex hormones would seem quite logical. The discovery of the fact that the composition of the serum lipids is different in the premenopausal woman as was shown in Table III would seem to indicate that the level of circulating estrogens was quite significant. In general, it can be seen from this that young men and young women have about equal concentrations of cholesterol, but that in young women the composition of phospholipid is higher, that the cholesterol-phospholipid ratios tend to be lower, and there is a greater percentage of the total

cholesterol in the form of alpha lipoprotein and a lower Sf 10-20 concentration. In contrast to this, it is remarkable that the lipid composition of plasma in men and women past the age of forty-five differs but little. With these facts in mind, it would seem possible that the mere presence of circulating estrogens in considerable quantities might affect the concentration and distribution of lipids.

The attempts at modification of the chemical composition of the plasma by the administration of estrogenic substances represents a new approach to the treatment of atherosclerosis. Although there have been several reports in the literature implying the significance of estrogens in the pathogenesis of atherosclerosis, reports on its' therapeutic trial and effects are relatively few.

One of the first reports on the clinical use of estrogens on atherosclerosis was that of Barr (39) and his co-workers at Cornell. A review of their work and that of several other similar investigations conducted by others will now be considered.

As subjects for their trial, survivors of myocardial infarction were chosen. To these patients, Estinyl (Estinyl Estradiol-Scherring) or Premarin (Conjugated Equine Estrogens-Ayrest) in oral dosages of 10,000 rat units were given. This amount was sufficient to produce, after two or three weeks, changes in the size of the breasts and a temporary loss of sexual desire and potency. They found in these subjects a response in the lipid pattern much greater than had been anticipated. In every instance there was an

increase in the percentage of cholesterol in the alpha lipoprotein fraction and a corresponding reduction in the percentage of cholesterol in the beta lipoprotein fraction. There was a marked fall in the cholesterol-phospholipid ratio of the whole plasma and in the fraction containing beta lipoproteins. In most cases there was also a fall in the concentration of total cholesterol in the plasma. The final values obtained were approximately those of young healthy men. When the medications were discontinued, the concentration and distribution of lipids returned promptly to their previous levels. These effects are further illustrated in Tables VI. and VII.

Table VI. Effect of Estrogen Administration on the Concentration and Distribution of Cholesterol in the Plasma of Survivors of Myocardial Infarction.

Stage of Treatment	Total Cholesterol mg. %	Cholesterol in Alpha lipoproteins		Cholesterol in Beta lipoproteins mg. %
		% of total	mg. %	
Before	282	11.3	32	250
After 1 week	283	16.7	41	243
2 weeks	270	17.5	47	223
3 weeks	260	19.0	49	211
4 weeks	251	21.0	53	198
5 weeks	247	22.0	55	191
6 weeks	225	25.4	57	168
7 weeks	222	25.0	56	166
8 weeks	216	26.0	56	160
9 weeks	210	27.1	57	153

Table VII. Effect of Estrogen Administration on the Cholesterol-Phospholipid Ratios of Plasma and Its Fractions in Survivors of Myocardial Infarction.

	Cholesterol-Phospholipid Ratios		
	Plasma	Alpha lipo-proteins	Beta lipo-proteins
Before estrogen administration	1.05	0.47	1.32
At end of estrogen administration	0.72	0.42	0.97

In this same investigation, it was shown that methyl testosterone will promptly reverse the effects of estrogens when given in large enough doses. It is interesting to note that they also found this neutralizing effect disappeared, and the estrogen effect was again realized as soon as the testosterone was discontinued.

Another similar investigation conducted on a group of twenty men with clinically proven coronary artery disease was conducted by Oliver and Boyd. (40) To this group they administered estinyl estradiol. They found that in doses greater than 0.2 mg. daily that significant changes in the plasma lipid pattern occurred. The average fall of total cholesterol in their group was about 25 per cent, beginning with levels of 314 +/- 55 mg. % and at the end of their investigation averaging 236 +/- 59 mg. %. The cholesterol-phospholipid ratio also was depressed, from 1.06 and 0.11 to 0.75 and 0.06. Side effects including gynecomastia, nausea,

dizziness, listlessness, fatigue and depression, loss of libido occurred in some of the subjects during the first two or three weeks. A subtile psychological feminizing change developed in one case. No overall change in the incidence or severity of effort, pain, or dyspnea was observed in this group of patients, however.

At the last meeting of the American Society for the Study of Arteriosclerosis, Katz and his associates gave their first interim report on the effects of estrogen therapy in males under fifty years of age with history of a previous single myocardial infarction. (41) Their study was conducted on sixty-nine patients. They were divided into two groups on a "double blind basis",-- estrogen treated and placebo. Analysis of these groups revealed then to be practically identical in regards to mean age, mean initial weight, mean duration after infarct when study was begun, mean duration of treatment, mean pre-treatment, plasma cholesterol levels, and mean pre-treatment plasma total cholesterol-lipid phsophorus ratios. Oral estrogen dosages were increased stepwise from an initial level of 1.25 mg. or 2.5 mg. of mixed conjugated equine estrogens (Premarin) to 4.0 to 10.0 mg. daily. They found that dosages of 1.25 to 4.0 mg. had no effect on the plasma total cholesterol levels. Higher dosages tended moderately to lower the cholesterol-lipid phosphorus ratio. They report that side effects including gynecomastia, loss of libido and impotence were induced by estrogens, but such effects only rarely resulted in discontinua-

tion of therapy.

In the placebo group of twenty-six patients under treatment for two months or longer, a second proved myocardial infarction had occurred in six, with three fatalities. In contrast, in the group of thirty-nine under estrogen treatment for two months or longer, no recurrence of myocarding infarction had occurred. It was admitted that neither the size of the groups nor the duration of treatment justifies any definative conclusions concerning the significance of the initial observations. However, they felt that continuation and expansion of this investigation would seem highly warranted.

Marett and Vivas (42) showed reductions in total lipids and total cholesterol following small daily doses of Premarin over a period of twenty-five weeks. Their subjects included twelve men who had survived a myocardial infarction, five others with miscellaneous conditions, and one woman who had survived a cerebral vascular accident. Gertler, Hudson, and Jost (43) found some increase in serum phospholipid level without change in the concentration of serum cholesterol in twenty-five patients who received daily doses of 500 mg. of diethylstilbesterol following bilateral orchidectomy for carcinoma of the prostate.

The only completely discordant results concerning the action of estrogens is in the report of Glass and associates. (44) In their study of sixteen men whose ages varied from fifty to

seventy years with benign prostatic hypertrophy and in fifteen women whose ages varied from forty-two to sixty-five years, all having post-menopausal symptoms, they found no evidence that estradiol in doses of 0.25 to 0.75 mg. orally daily for periods of two to four months influenced the level either of the serum lipids or low density proteins. They suggested that giving higher doses or by giving it parenterally may have produced different results.

All of these observations, with the exception of the latter, on the action of sex hormones may be regarded as important for several reasons. First, they show that the concentration and distribution of lipids are subject to hormonal influence. Second, they indicate that there may be a chemical reason for the apparent partial immunity of young women to the complications of atherosclerosis. Thirdly, they demonstrate that the abnormal patterns of lipid distribution encountered in most survivors of myocardial infarction and in certain other atherosclerotic conditions can be manipulated by the use of estrogens, and can be changed to values more appropriate to young men and women. Lastly, the action of methyl testosterone suggests that it might not be a desirable medication in patients who have extensive atherosclerotic disease. (17)

The incidence and severity of atherosclerosis in estrogen-treated males and in females with a hypoestrogenic or hyperestrogenic state was analyzed by Rivin and co-workers. (42) Clinical and autopsy statistics were compiled in groups of patients who

demonstrated these deviations from their normal estrogen supply. Hyperestrogenism in males was studied in patients with carcinoma of the prostate who were treated with estrogens. Hyperestrogenism in females was considered to be present in women with carcinoma of the breast. The hypoestrogenic state was studied in a group of surgically castrated females. The incidence and severity of atherosclerosis in these patients were then compared with that in similar groups who were apparently normal with reference to their estrogen supply. Results from these studies demonstrated: (a) an apparent diminution of coronary atherosclerosis in males treated with large doses of estrogen; (b) a significant increase in atherosclerosis, especially in the coronary arteries, in women who have had their estrogen supply reduced by castration; (c) an incidence of severe atherosclerosis in the hyperestrogenic female, even less than that of the normal female.

Apparently the estrogen level necessary to produce an antiatherogenic effect is fairly high, for in patients with carcinoma of the prostate receiving 5 mg. of diethylstilbesterol daily, convincing evidence of estrogenic protection against atherosclerosis was lacking. On doses of 75 mg. or more daily, however, the degree of atherosclerosis found was less than in the normal male.

The implications of these clinical investigations would thus tend to support the experimental evidence derived from animal studies which suggest that females have less atherosclerosis than

males because ovarian secretions in some way protect them from this disease.

SUMMARY

Atherosclerosis is one type of arteriosclerosis. It is by far the most devastating form of arterial disease, for it is the chief cause of coronary artery disease and cerebral vascular accidents. The walls of the involved arteries show fatty degeneration and/or infiltration of lipids within and beneath the intima with resultant plaque formation. Marked atherosclerotic lesions have been found in very young people at autopsy and are often found to be minimal in the very aged which would tend to disprove the generally accepted theory that atherosclerosis is an inevitable by-product of aging. Certain diseased states including diabetes mellitus, hypothyroidism, biliary obstruction, the nephrotic syndrome, and essential xanthomatosis are all associated with severe, premature atherosclerosis. All of these entities are associated with alterations of cholesterol-lipid-lipoprotein metabolism. An overwhelming mass of evidence all points to this alteration as being the key factor in the pathogenesis. Anatomical and physiological factors concerning the vessel wall are undoubtedly in some way also involved. The similarity of the initial relative composition of the lesion of the vessel wall and the plasma support the view that the source of the vessel wall lipid is the plasma. The plasma lipid pattern of the atherosclerotic individual generally shows alterations in the following ways: (1) the total cholesterol is elevated, (2) there is an increase in the cholesterol/

phospholipid ratio, (3) elevation of Sf 10-20 (Gofman) bodies, (4) an elevation of beta lipoprotein and a decrease in alpha lipoprotein. The general trend of current investigational work tends to place the greatest pathological significance on the latter.

The relative infrequency of coronary artery disease in premenopausal women and the increased frequency of this disease in bilateral oophorectomized women suggests that estrogens may in some way exert some inhibiting effect on atherogenesis. Analysis of the plasma lipid composition in younger women reveal it to be more similar to that of newborn infants and mammals who are relatively free of the disease. Presumably the chief effect of estrogens is on the lipoprotein levels, causing an elevation of the alpha fraction and reduction of the beta fraction. The liver has been suggested as the site of action, since most of the plasma proteins are both synthesized and degraded by this organ. This action may be mediated through the anterior pituitary which in turn influences the thyroid and adrenal glands.

Most of the animal experimental work has been done on the chick primarily because they develop atherosclerosis spontaneously and because of the ease with which it may be produced by a cholesterol-fed diet. In this animal, it was noted that diffuse lipoid infiltration of the coronary arteries which had been induced by a cholesterol-fed diet regressed spontaneously at the time of sexual maturation. Other similar experiments have shown actual

inhibition and regression of coronary atherosclerosis by parenteral administration of estrogens with return of previously elevated total cholesterol/phospholipid ratios to normal. Gonadectomized female and intact male chicks uniformly developed coronary atherosclerosis on a cholesterol-fed diet, whereas, intact females the incidence was only 40 per cent.

In humans it has been proven that it is possible to alter the plasma lipid composition of atherosclerotic individuals to approximately that of healthy young men and women by the administration of estrogens. Most of the subjects of this investigational work have been survivors of a myocardial infarction. Although the size of the groups studied thus far are too small and the duration of treatment too short to justify any definitive conclusions, it appears that the administration of estrogens may prove to be of value in the treatment of atherosclerosis.

CONCLUSIONS

1. Atherosclerosis is a disease of altered cholesterol-lipid-lipoprotein metabolism, which is not an inevitable or necessarily an irreversible by-product of aging.

2. The plasma lipid composition and distribution of man, who is prematurely subject to the complications of atherosclerosis, differs significantly from that of premenopausal women and other mammals who are relatively immune to this disease.

3. It is possible to alter the abnormal lipid composition of the plasma of atherosclerotic individuals to that more closely resembling relatively immune subjects by the administration of estrogens.

4. The implications of animal and clinical investigations, although not yet definitely established, indicate that the administration of estrogens may prove to be of value in the treatment of atherosclerosis, and certainly the continuation and expansion of this work would seem highly warranted.

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