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# The Effect of estrogens on coronary atherosclerosis

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# THE EFFECT OF ESTROGENS ON CORONARY ATHEROSCLEROSIS

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## I. Introduction

For many years it has been noted that coronary atherosclerosis was much more pronounced in males than in females. This difference was adequately demonstrated by Ackerman and White's autopsy findings in which they compared 600 consecutive male hearts with 600 consecutive female hearts and found that in comparable age groups there was a great deal more atherosclerosis in the coronary arteries of males than of females.

This difference has stimulated considerable interest because if the factor which produces this difference could be isolated a possible therapeutic aid in the treatment of coronary heart disease might be forthcoming.

In this paper data has been collected from the literature which demonstrates, both from animal experimentation and clinical observation on human subjects, that estrogen may be this factor which protects the coronary arteries of premenopausal women.

II. Experimental Evidence

The basis on which the estrogen induced regression of coronary atherosclerosis is established is the cholesterol concept of atherogenesis. The basic tenet of this concept is that without an altered lipid-cholesterol metabolism little or no atherosclerosis will develop regardless of any other alterations in the arterial wall including senescence. There has been considerable experimental evidence to show that this concept is valid.

Prior to 1908 all efforts to produce atherosclerotic lesions in experimental animals had failed. In 1908 a group of investigators found that rabbits fed meat, milk or eggs developed atherosclerotic lesions and it was not long after that Anitschkow demonstrated that cholesterol was the factor responsible. Since that time experimental atherosclerosis has been produced by no other means (except for avian stilbesterol induced atherogenesis, a lesion secondary to endogenous endocrine-stimulated hypercholesteremic hyperlipemia). (1)

A number of objections were raised to the use of the rabbit as an experimental animal for this work. First the rabbit is a herbiverous animal which does not normally ingest cholesterol. On its usual diet it does not develop atherosclerotic vascular lesions and furthermore, until the last decade, only limited and indifferent success was obtained in attempting to

induce gross atherosclerotic lesions in species other than the rabbit. In consideration of this fact many were questioning whether the experimental work in the rabbit was relevant to the clinical lesions seen in man. For this reason Katz and his group chose the chick as the experimental animal of choice and most of the experimental work on estrogen as prophylaxis against and treatment for coronary atherosclerosis has been done on the chick. Like man it is omniverous and spontaneously develops atherosclerotic lesions of the great vessels. Moreover it was demonstrated in early studies (2) that cholesterol feeding led to hypercholesterolemia and on to atherosclerosis of the major vessels including the coronaries.

Studies in the chick demonstrated a close correlation between the level of hypercholesterolemia and aortic atherosclerosis. Because a number of recent investigators had produced evidence from man that the cholesterol-phospholipid (C/P) ratio may be a big factor in atherogenesis, Stamler and Katz also tried to correlate C/P ratios with aortic atherosclerosis in the chick and found no correlation. Up to this time the majority of the experiments had used the aorta as the index of atherosclerosis because of the relative ease of examining it. However, during these experiments on the relation to C/P ratio Pick, in the same laboratory (Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital in Chicago, Ill.), examined the coronary arteries of these chicks and found a

definite correlation between C/P ratio and coronary atherosclerosis. This pointed up the fact that different vascular beds may vary widely in their resistance to the atherogenic process and that severe atherosclerosis may be present in one region while others are spared.

Pick and her associates (3) later found that by administering estrogens to the cholesterol fed chicks the coronary arteries were protected from atherosclerosis even though the aorta developed atheroma as before. This protection of the coronary arteries seems to be related to associated changes in C/P ratios.

As had previously been demonstrated chicks fed cholesterol diets developed a hyper-cholesterolemia with a resultant increase in C/P ratio. When the estrogen was administered in combination with the cholesterol a concurrent elevation of the phospholipids are thus elevated but the ratio remains about normal (12-13). Birds with a C/P ratio of less than 20 were routinely free from coronary atherosclerosis. Those with a higher ratio were not protected. (4)

A striking illustration of the fact that the C/P ratio and not the hypercholesterolemia was the cause of the lesion was seen in two birds taken out of the same group of chicks. One estrogen treated bird had a cholesterol level of 1800 mgm. per 100 c.c. but had a C/P ratio of only 16 and had no coronary lesions; another non-estrogen treated bird had a cholesterol

level of only 460 mgm. per 100 c.c. but a relatively high C/P ratio of 25. It had distinct coronary atherosclerosis (5).

After showing that estrogen protected the chicks from coronary atherosclerosis this group went on to test the effect of estrogens on birds with experimentally developed coronary atherosclerosis in the hope that a therapeutic value could be established. Fifty cockerels were fed commercial mash until they were seven weeks old. During the next five weeks the mash was supplemented with 2% cholesterol plus 5% cottonseed oil (2 CO diet). At the conclusion of this period ten birds were sacrificed, all of which showed marked aorta and coronary atherosclerosis. The balance of the birds were divided into four groups of ten birds each. Group I and II both received the 2 CO diet for a period of three weeks more with group II receiving additionally 1 mg. of estrogen per day. Groups III and IV received the 2 CO diets for additional period of eight weeks for a total of twenty weeks. During the last five weeks of this period group IV received 1 mgm. of estrogen per day. When these four groups of birds were sacrificed the ones which received estrogens all showed a marked regression of lesions. Those in group IV, which received estrogens for five weeks were almost completely free from lesions. In fact one-third of the birds had no lesions remaining and the remaining had only an occasional vessel with a residual plaque evident. There

was a clearing of both lipidosis and fibrosis from the vessel wall. (6) As had been noted in previous experiments, however, the estrogen had no effect on the lesions of the aorta. (7) It was noted that in birds from groups III and IV that C/P ratios waried from an average of 56.7 in the group III untreated birds to 12.0 in birds of group IV which received estrogen.

The mechanisms of estrogen-induced regression of coronary atherosclerosis remain obscure. It is conceivable from the peculiar histologic picture of the lesions showing partial regression that estrogen causes a lipophage activity which causes the lipids to migrate to the adventitia and perivascular tissue. There is also the possibility that estrogen may decrease the permeability of the endothelium for lipids. The key factor may be the effect of this hormone on plasma lipid-lipoprotein complexes, but these mechanisms are all merely speculations and await additional evidence to confirm or deny their validity.

Goffman and his associates had shown that there was an association of lower density molecules with clinical atherosclerosis. On ultracentrifugal analysis it was found that normal young growing cockerels exhibited a serum lipoprotein pattern characterized by uniform presence of molecules of class $S_f$ 3-8. When cholesterol was added to the diet there was an immediate increase in lower density molecules classes $S_f$ 10-100 associated with a hypercholesterolemia and aorta and coronary atherosclerosis. When estrogen was used on these birds it was found that

there was both a prophylactic and a therapeutic effectiveness against coronary atherosclerosis. However, estrogen had no effect on the plasma lipid-lipoprotein patterns. The protection of the coronary arteries remained when testosterone was added to the estrogen therapy but this produced an even higher level of serum lipoproteins of classes $S_f$ 3-8, $S_f$ 10-20 and $S_f$ 20-100 and greater.(8) This demonstrated that the efficacy of estrogen was not in its effect on the macro-molecules which are evident in atherosclerosis.

Following up its lead in the use of estrogens the group at Michael Reese Hospital decided to try gonadectomy on experimental chicks. A group of 60 male and 60 female chicks were used and the age of thirteen weeks were started on a diet of mash supplemented with 1% cholesterol and 5% cottonseed oil (1 CO diet) which was continued for five weeks. Three weeks before they were started on the 1 CO diet 30 males and 30 females were gonadectomized. These four sets of 30 chicks each were then subdivided into three groups of ten chicks each. One group was given 1 CO only, another group 1 CO plus testosterone and the third group 1 CO plus estrogen. When these birds were sacrificed it was found that the degree of hypercholesterolemia was comparable in all of the birds. The incidence and degree of aorta atherosclerosis was also similar in all groups. However, the incidence of coronary atherosclerosis in intact untreated females was low in comparison with gonadectomized untreated

females and intact untreated males. All of the birds that received estrogen whether male, female, intact or gonadectomized were uniformly free from coronary lesions. (9)

Because of the femininizing effect of estrogen, which would be a drawback in its clinical use in the treatment of coronary atherosclerosis, it was decided to try giving androgen or chorionic gonadotropin in conjunction with it. The experimental chicks were fed the usual cholesterol diet to produce the coronary lesions and were then divided into groups each receiving one of the following treatments 1.) estrogen 2.) testosterone 3.) testosterone plus estrogen 4.) chorionic gonadotrophin or 5.) chorionic gonadotrophin plus estrogen. It was found that testosterone or chorionic gonadotrophin alone had no effect on cholesterolemia, phospholipemia or C/P ratios and likewise had no effect on coronary atherogenesis. However, in combination with estrogen they produced a prophylactic inhibition of coronary lesions comparable to that of estrogen alone.(10)

The question had been previously raised as to whether the relative susceptibility of the human male to coronary atherosclerosis was due to absence of estrogen or presence of androgen or to both (i.e., a low ratio of estrogen to androgen.) This study showed coronary atherogenesis proceeded unabated whether the chick was hyperandrogenic, euandrogenic or hypoandrogenic. Therefore, the presence of estrogen appears to be the factor which is essential for the antiatherogenic activity.(11)

In further studies Katz and his group treated cockerels with estrogen for three weeks prior to cholesterol feeding and discontinued it with the advent of cholesterol feeding. It was found that the resultant plasma hyperlipemia was typically of the cholesterol induced type with no evidence of an estrogen effect. However, partial inhibition of coronary atherosclerosis apparently occured.(12)

Due to the depression of atherosclerosis in pre-menopausal women, it was decided to assess the influence of endogenous estrogen secretion on atherogenesis in egg-producing hens. In this experiment four groups of chickens were used 1.) intact egg-laying hens 2.)oviduct-ligated hens (oviducts were ligated in order to prevent cholesterol loss through egg production) 3.) intact roosters 4.) gonadectomized roosters. It was found that the first two groups were free from coronary atherosclerosis while the latter two groups developed it. It was thus demonstrated that endogenous estrogen secretion at the physiologic level was adequate to protect the coronary vessels of egg-laying hens against atherosclerotic change.(13)

III. Clinical Evidence

In addition to the experimental evidence which has been accrued there has been considerable clinical evidence which points to the estrogenic effect in inhibiting coronary atherosclerosis.

Ackerman, Dry and Edwards made a study of 600 female hearts at autopsy.(14) One-hundred consecutive hearts were chosen in each of the six decades in the age group 30 through 89. These were compared with 600 male hearts taken in the same age distribution which were studied by White.(15) It was found that in women the degree of sclerosis increased steadily from the fourth to the eighth decade but leveled off in the ninth decade. It was also noted that the degree of coronary atherosclerosis was much more severe in males; however, after the seventh decade the average grade of sclerosis in men was only 13 to 17% greater than in women. There is a marked increase in between the fifth and sixth decade and a similar marked increase in women between the seventh and eighth decade.

The fact that the greatest increase in coronary atherosclerosis, as well as the leveling off following the increase, came about two decades later in the female than in the male caused investigators to wonder whether early menopause would change the female curve toward that of the male. If this was so it would indicate that estrogens were involved rather than an

anatomic difference between male and female hearts. Wiest, Dry and Edwards (16) consequently studied 49 hearts of women who had undergone bilateral oopherectomy during the childbearing age 2 to 42 years before death. They found that there was no significant increase in atherosclerosis until at least five years following castration. In considering the number of women in each age group in which severe atherosclerosis had developed in the coronary arteries they found that the percentages turned out to be much greater than in control women of the same age groups and more closely approached that of control men.

Another bit of evidence in favor of the efficacy of estrogens was pointed out by Wuest.(17) In a series of 59 hearts of men with cirrhosis of the liver it was shown that they developed a lower degree of atherosclerosis than the control series. It had been previously reported that men cirrhosis of the liver fail to inactivate normally occuring estrogens.

There is also evidence to show that estrogens have an effect on cholesterol-phospholipid (C/P) ratios in humans as well as in experimental animals. Eilert (18) in a series of eleven female patients receiving estrogens noted that in all cases there was a sharp reduction in the ratio of total cholesterol to lipid-phosphorus during periods of estrogen administration. Another group at the Hollywood Presbyterian Hospital in Los Angeles (19) also noted that C/P ratios returned to normal when estrogens were administered. This return was usually ac-

complished by an increase in the phospholipid level. Barr's group (20) in doing fractionation studies on blood lipids using Cohn's technique $10^2$ found that there was a difference between young men and women in distribution of cholesterol in the plasma. In young women the cholesterol of alpha lipoprotein constituted 35% of the total while in young men the values were about 25%. However, analyses of men and women after the age of 45 failed to demonstrate any considerable difference between the sexes. Thus in the childbearing years there is a lowering of the C/P ratio in females which is apparently due to the effects of estrogens.

Current thought and experiments indicate that the stability of emulsions of lipids in plasma is to some extent influenced by the relative concentration of cholesterol and phospholipids and in such a manner that increase in C/P ratios diminish solubility while reduction in the ratios increase it. They speculate that estrogens may stabilize the plasma by increasing the concentration of alpha lipoproteins with their low C/P ratios at the same time that it depresses the concentration of beta lipoproteins with C/P ratios more than twice as great.

## IV. Summary

Experimental evidence for the estrogen induced regression of coronary atherogenesis was obtained by feeding a high cholesterol diet to chicks. Reasons for the use of the chick rather than the rabbit as the experimental animal have been presented.

A close correlation between the level of hypercholesterolemia and aortic atherosclerosis was noted in the chick but no correlation between cholesterol-phospholipid (C/P) ratios and aortic atherosclerosis could be found. This was disappointing as evidence from man had indicated that C/P ratio might be a big factor in atherogenesis. However, before the experiments were concluded it was found that a definite correlation between C/P ratio and coronary atherosclerosis was present. Thus it became apparent that different vascular beds may vary widely in their resistance to the atherogenic process.

It was demonstrated that birds fed estrogens along with a high cholesterol diet showed an elevation of phospholipids along with the hypercholesterolemia. This maintained the C/P ratio at near normal and protected the coronary arteries from atherosclerosis even though the aorta developed atheroma as before.

The next group of experiments showed that estrogens not only protected the coronary arteries but actually caused

a regression of previously established coronary atherosclerotic lesions with a clearing of both lipidosis and fibrosis from the vessel wall.

The mechanisms of estrogen-induced regression of coronary atherosclerosis are not clear. One thought is that estrogen causes a lipophage activity which causes the lipids to migrate to the adventia and perivascular tissue. Another idea is that estrogen may decrease the permeability of the endothelium for lipids. Still another factor may be the effect of this hormone on plasma lipid-lipeprotein complexes. These are all merely speculations, however, and must await further evidence to confirm or deny their validity.

Because Goffman and his associates had shown that there was an association of lower density molecules with clinical atherosclerosis ultracentrifugal analysis was performed on birds which had received estrogens. It was found that the efficacy of estrogen was definitely not in its effect on the macro-molecules, which are evident in atherosclerosis.

Experiments also showed that estrogen retained its effectiveness in counteracting coronary lesions even though the femininizing effect of estrogen was counterbalanced with androgen. It was also shown that androgen itself had no effect on atherogenesis either advantageously or adversely. Also demonstrated was the fact that endogenous estrogen secretion at physiological levels was adequate to protect the coronary vessels

of egg laying hens against atherosclerotic changes.

From man, too, there has been much evidence to show that estrogen has an inhibiting effect on coronary atherosclerosis. Comparison of male and female hearts indicate that the greatest increase in coronary atherosclerosis as well as the leveling off following the increase occurs two decades later in the female than in the male. It was found that in bilaterally oopherectomized women the incidence of coronary atherosclerosis was much higher than in control women of the same age groups and more closely approached that of control men. It was further noted that men with cirrhosis of the liver, which prevented them from inactivating normally occuring estrogens, had a lower incidence of coronary atherosclerosis than did control men.

It has been noted clinically as well as experimentally that the administration of estrogens to patients results in a sharp reduction in C/P ratios. This reduction of C/P ratios apparently increases the stability of emulsions of lipids in plasma. It is speculated that estrogens may stabilize the plasma by increasing the concentration of alpha lipoproteins with their low C/P ratios at the same time that it depresses the concentration of beta lipoproteins with C/P ratios more than twice as great.

V. Conclusion

It has been shown both experimentally and clinically that estrogen has a beneficial effect in both prophylactic and therapeutic treatment of coronary atherosclerosis. However, the fact remains that males, who are most susceptible to this condition, are not likely to accept the femininizing features which would accompany long term use of the hormone. It is very possible, however, that in humans, as in experimental cockerels, that a combination of estrogen and androgen which will maintain the beneficial results while masking the femininizing effects may prove feasible. Another possibility is that research may be able to break down the estrogen molecule in such a way that the beneficial effects will be retained and the femininizing effects removed.

These developments are still in the future, in the realm of things to be hoped for. However, it is gratifying to know that advancements are being made which may someday enable the medical profession to treat this disease which continues to take a tremendous toll of humanity.

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