Effects of Estrogen Therapy on Hormonal Functions and Serum Lipids in Men with Coronary Atherosclerosis

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Middle-aged men with coronary atherosclerosis were studied before and at intervals after initiation of estrogen therapy over a 12-month period. Serum lipid studies during treatment revealed consistent increases in phospholipid and α-lipoprotein cholesterol and decreased β/α-lipoprotein cholesterol ratios. Concomitant endocrine studies demonstrated the expected testicular depression without apparent detriment to thyroid or adrenal function.

PREVIOUS investigators have demonstrated alterations of the serum lipid patterns with estrogen therapy in coronary atherosclerosis, both in experimental animals and in patients with this disease. In 1949 Eillert\(^1\) noted that estrogen administration to female patients produced a rise in serum lipid phosphorus and a fall in cholesterol, with a consequent decrease in the cholesterol-phospholipid (C/P) ratio. Later, Katz and Stamler\(^2\) showed that estrogens were effective, prophylactically and therapeutically, against cholesterol-induced coronary atherogenesis in cockerels. Barr, Russ, and Eder\(^3\) gave estradiol or Premarin to male survivors of myocardial infarction and showed that this therapy altered the abnormal serum lipid patterns toward normal adult human values. Similarly, Steiner, Payson, and Kendall\(^4\) reported that administration of ethinyl estradiol to 5 male patients and 3 control patients resulted in either a decrease in serum cholesterol or an increase in phospholipid, with resulting decrease in C/P ratio in all cases. Before estrogen therapy could be accepted for more extensive clinical trial, a study was indicated not only of the effects of estrogen on serum lipids but also on various endocrine functions when given over long periods and in large dosage to men.

METHOD AND MATERIAL

A group of 51 men with myocardial infarction have been treated continuously with estrogen for periods of 6 to 48 months (average, 24 months). Of these, 20 middle-aged males (mean age, 50.5 years) were studied before and at intervals up to 1 year after initiation of therapy with 5 to 20 mg. (average, 10 mg.) daily of oral mixed conjugated estrogens (Premarin). This drug was selected for its freedom from production of nausea and vomiting, which permitted long-term administration. Twenty similar patients (mean age, 48.5 years) received placebo tablets and served as a group for comparison. The estrogen-treated and control groups were comparable as to incidence of angina pectoris, myocardial infarction, congestive heart failure, hypertension, and diabetes.

The effects of estrogen therapy were explained to both the patient and his wife, and their consent was obtained for the treatment. This precaution minimized any subsequent psychologic difficulties.

Serum cholesterol was determined with the acid-iron reagent of Zlatkis, Zak, and Boyle\(^5\) after hydrolysis and extraction according to Abell, Levy, Brodie, and Kendall\(^6\). In our hands, this technic yielded cholesterol values averaging 53 ± 1 mg. per cent lower than did the direct method of Zlatkis and co-workers (N = 350; s.d. = 22 mg. per cent). The high intrinsic sensitivity of the acid-iron reaction permitted convenient, routine cholesterol analyses on 0.2 ml. of serum.

Lipid phosphorus was estimated by a micro-method involving re-extraction with petroleum ether\(^7\) of a dried ethanol-ether (3:1) extract of 0.2 ml. of serum, removal of solvent, wet-ashing ac-
according to Youngberg and Youngberg, and determination of liberated inorganic phosphate according to Fiske and SubbaRow. Lipid phosphorus X 25 was taken as serum phospholipid.

The "α-" and "β-" lipoprotein fractions of serum were separated by zone electrophoresis, by means of the filter paper "sandwich" technic described by Kunkel and Slater. After migration the papers were cautiously air-dried below 60 C., and narrow strips were taken for staining with bromphenol blue (proteins) and oil red O (lipids). The areas on the unstained strips corresponding to the α-lipid (albumin plus α₁-globulin) and β-lipid (β-globulin) zones were removed separately, the lipids were eluted with chloroform-methanol (3:1), the solvent was removed in vacuo, the residues were re-extracted with petroleum ether, and the cholesterol content of aliquots of the final extracts was determined with the acid-iron reagent.

For fractional analysis of urinary 17-ketosteroids, extracts of aliquots of 48-hour urine specimens were prepared by sequential hydrolysis of conjugates with mammalian β-glucuronidase, continuous ether extraction of residual urine made 2N in sulfuric acid, and boiling in 2N acid, as recommended by Lieberman, Mond, and Smyles. Separate ether extracts were prepared after each hydrolysis, combined, washed with 0.1 N NaOH and water to neutrality, and evaporated to dryness under nitrogen. 17-Ketosteroids were fractionated by gradient elution chromatography with ethanol-benzene on moist alumina, according to the method of Lakshmanan and Lieberman. It should be noted that this technic does not separate dehydroepiandrosterone* from etiocholanolone§ from their respective α₃-β₁-unsaturated derivatives. However, artifact formation was minimized by the scheme of hydrolysis and extraction employed, and qualitative sulfuric acid spectra from 220-460 nm have indicated the presence of only minor amounts of these companion substances in material eluted from the chromatograms.

Testicular biopsies were performed under local anesthesia on 10 individuals before initiation of estrogen therapy, and again after 1 month and 12 months of therapy. Specimens were fixed in 10 per cent formalin solution and stained by conventional and histochemical technics. The results of the histochemical studies will be reported elsewhere. The histologic studies were performed independently.

Thyroid function was evaluated by the oral administration of a tracer dose of 50 μc. of carrier-free 1₃₁I. The measurement of radioactivity over the thyroid gland was taken 24 hours later. Four lead-shielded copper cathode tubes were spaced 90° apart at the circumference of a 90 cm. diameter horizontal circle and connected in parallel to a scaler. The patient was seated so that his thyroid gland was located in the center of this circle, where there was an 18 cm. diameter sphere in which counts varied only within 2 per cent. Background counts varied between 350 and 400/min., allowing all studies to be made at levels of at least 2.5 X background.

Results

The clinical effects of estrogens on males are well known. Changes in pigmentation, skin texture, and growth and distribution of hair have been described, as well as gynecomastia and testicular atrophy. In those patients whose sexual potency was initially intact, this function gradually decreased, disappearing within 1 to 24 months in all patients on a dose of 10 mg. or more of Premarin daily. Some degree of potency has returned to 3 patients who had received 10 mg. daily for 6 to 24 months before withdrawal of therapy. There has been no evidence to date of a return of fertility in these patients, although circumstances of age prevent an adequate appraisal. The most distressing effect to the patients was gynecomastia, which was minimal (tenderness and slight swelling) in most of the patients and moderate in only 2. The tenderness usually subsided within a few months. An unexpected observation was the growth of new scalp hair in 4 bald patients. The only cardiac complication to the therapy was the appearance of pulmonary edema in 2 patients, which did not respond to digitalis and diuretics, but did subside promptly on omitting the estrogen.

It is clear that the control group showed no significant alteration in serum cholesterol (fig. 1) or phospholipid (fig. 2) during the year of observation. The estrogen-treated group showed a decrease of cholesterol, of borderline significance statistically, by the end of 3 months (fig. 1). Figure 2 shows the strikingly significant increase of phospholipid in the treated group, from 207 mg. per cent to 248 mg. per cent at 3 months. This increase was maintained to 12 months. Figure 3 demonstrates that the C/P ratio in the estrogen-treated group, initially 1.30, was significantly decreased to 1.14 at 1 month, further decreased at 3 months to 0.96, and maintained thereafter to 1 year. The
The distribution of serum cholesterol in the \( \alpha \)- and \( \beta \)-lipoprotein fractions as per cent of total cholesterol is shown in figure 4. The control group showed no significant changes during the 1-year period, despite the apparent decrease

Fig. 1. Mean serum cholesterol levels in estrogen-treated and control groups observed for 12 months. The figures indicate mean values and standard errors of these means.

Fig. 2. Mean serum phospholipid levels in estrogen-treated and control groups for 12 months.

Fig. 3. Serum C/P ratios in estrogen-treated and control groups for 12 months.

Fig. 4. Distribution of cholesterol in serum \( \alpha \)- and \( \beta \)-lipoprotein fractions (separated by zone electrophoresis) in estrogen-treated and control groups for 12 months. Lipoprotein cholesterol calculated as per cent of total cholesterol in each fraction.

Fig. 5. Serum \( \beta/-\alpha \) -lipoprotein cholesterol ratios in estrogen-treated and control groups for 12 months.
in the β-lipoprotein fraction. On the other hand, the estrogen-treated group showed a striking rise in α-lipoprotein cholesterol from 10 to 15 per cent in 1 month, a further increase to 20 per cent in 3 months and subsequent maintenance near this level. The cholesterol in the β-fraction showed a significant fall from 56 to 45 per cent in 1 month ($p < 0.01$) that was unchanged thereafter. However, the latter results are of dubious validity, since the control group showed a similar but not significant fall from 58 to 50 per cent in 1 month ($p = 0.1$).

Figure 5 shows the effect of these reciprocal changes upon the ratio of β- to α-lipoprotein cholesterol. There was no significant change of this ratio in the control group, while the ratio in the estrogen-treated group had dropped sharply by 1 month, from 6.8 to 3.2, and was maintained thereafter near 3.0. It is clear that this effect was primarily due to the striking increase in the α-lipoprotein cholesterol fraction.

Figure 6 shows the changes observed in urinary 17-ketosteroids. It will be seen that in all instances the changes observed in the control group did not differ significantly from zero. This was also true of the total 17-ketosteroid output in the estrogen-treated group. However, the daily excretion of dehydroisoandrosterone was increased during estrogen therapy from 0.8 to 1.6 mg., while the excretion of androsterone and of etiocholanolone was

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**Fig. 6.** Alterations in urinary 17-ketosteroid excretion patterns during 1 year of estrogen treatment. Pretreatment levels and initial values in control group adjusted to zero line.

**Fig. 7.** Mean absolute urinary 17-ketosteroid excretion levels, mg./24 hours, in control and estrogen-treated groups, the latter shown prior to and during therapy.
markedly depressed (by 37 and 35 per cent, respectively). The 11-oxygenated ketosteroids remained essentially unchanged at approximately 1.5 mg./24 hours. Figure 7 shows the absolute values in milligrams per 24 hours of the total and component ketosteroids recovered after chromatography. The average daily 17-ketosteroid output in the control group was 14.8 mg. and in the treatment group 13.3 mg. before therapy. This remained relatively constant (12.8 mg.) during estrogen treatment.

Histologic studies of testicular biopsies prior to estrogen therapy (fig. 8A) revealed normal Sertoli cells, spermatogenesis, and Leydig cells. Serial biopsies during estrogen therapy showed suppression of all cellular activity of the testicle. At 1 month of treatment (fig. 8B) there was failure of sperm development, with subsequent suppression of spermatocytogenesis until only a few spermatogonia remained. At this time many tubules were lined only by Sertoli cells. Concomitantly, the tunica propria was thickened by a deposition of collagen and elastic fibrils. After 12 months of therapy
(fig. 8C) the tubules were converted into acellular fibrous cords. Regressive changes in the interstitial cells accompanied this tubular atrophy. The regressing cells diminished in volume, tended to become spindle-shaped, and the cytoplasm lost the coarse granulation and vacuolization presumed to be evidence of secretory activity; a few cells contained coarse granules of tan pigment or occasionally a crystalloid. In the final stage, Leydig cells were not identifiable and many collagen fibrils occupied the interstitium.

Table 1 shows the effect of estrogen therapy for 1 year on the 24-hour uptake of I\(^{131}\). Although there was a suggestive increase of the uptake in the estrogen-treated group from 35 to 39 per cent, this change did not prove to be statistically significant (\(p = 0.3\)).

### Discussion

Since gynecomastia proved to be somewhat distressing, especially in the younger age group, surgical removal of the rudimentary male breast glandular tissue has been performed before institution of estrogen therapy in the more recent cases studied. This is a simple procedure done under local anesthesia with replacement of the cutaneous nipple and areola. An excellent cosmetic effect has been achieved, and no breast hypertrophy has followed this procedure. It eliminates the psychologic effects of gynecomastia and avoids any possibility of malignant disease of the breast from estrogen stimulation.

In 3 patients in whom the loss of sexual potency was especially distressing the treatment was stopped. The serum lipids of 1 of these patients promptly reverted to abnormal levels.

It should be noted that in spite of prolonged estrogen therapy the basic masculine psychologic traits of these patients have remained intact.

The development of acute pulmonary edema noted in 2 patients was thought to be due to the sodium- and water-retaining effect of estrogens.\(^{16}\)

The serum lipid data presented are in general agreement with those of Eilert,\(^{17}\) Barr,\(^{18}\) and Gertler, Garn, and Lerman.\(^{19}\) For example, the mean serum cholesterol level in untreated men with myocardial infarction was reported by Barr to be 250 mg. per cent, and by Gertler and his associates to be 286 mg. per cent. The mean initial value in the present study was 265 mg. per cent. The chief difference from those studies is that the present phospholipid values were lower. This difference is ascribed to the petroleum ether purification employed in this work. It is of interest that Hack\(^{20}\) has reported that approximately 10 per cent of the serum phosphorus soluble in ethanol-ether is insoluble in petroleum ether but soluble in water. Similar results with ultracentrifugal fractions of serum have recently been reported by Havel, Eder, and Bragdon.\(^{21}\) The relatively lower phospholipid values of the present study are reflected in the higher C/P ratios.

By paper electrophoresis, significant changes in the percentage of cholesterol in the \(\alpha\)- and \(\beta\)-fractions of the lipoproteins were demonstrated during estrogen therapy. It was found that approximately 75 per cent of the total serum cholesterol was recovered in these fractions. In studies now in progress, individual sera have been submitted to zone electrophoresis and ultracentrifugation at a solvent density of 1.063. The \(\beta\)/\(\alpha\)-lipoprotein cholesterol ratios determined by the 2 technics have shown close correspondence. These studies will be reported elsewhere. The change in \(\beta\)/\(\alpha\)-lipoprotein cholesterol ratios was very similar to that reported by Barr using the Cohn microfractionation method no. 10.\(^{10}\) Barr's ratios changed from 7.8 to 3.7 in 4 weeks and the ratios presented here from 6.8 to 3.2 in 4 weeks. These represent identical decreases of 53 per cent.
It is noteworthy that despite the changes in excretion of individual 17-ketosteroids during estrogen therapy, there was no significant alteration in the mean output of total 17-ketosteroids. The excretion of dehydroisoandrosterone, presumably an "in-process" adrenal metabolite (Lieberman and Teich), was increased during estrogen therapy. Similar data have been presented by A. M. Robinson. The decreased excretion of androsterone and etiocholanolone is readily explained on the basis of diminished testosterone production by the Leydig cells of the testes. The serial testicular biopsy studies offer histologic confirmation of this finding. The relative constancy of excretion of the 11-oxygenated ketosteroids, metabolites of adrenal cortical origin, suggests that adrenal cortical function was not suppressed during estrogen therapy.

Thyroid function, as measured by the 24-hour uptake of a tracer dose of I, was not significantly altered after 1 year of estrogen therapy with the present dosage. Although this seems at variance with the data of Engstrom and Markardt, it should be noted that these investigators administered relatively large doses of diethylstilbestrol (75 to 100 mg. daily) to the 12 men studied. The serum lipid changes observed in the present study, therefore, cannot be ascribed to increased thyroid function.

Insufficient time has elapsed for a mature evaluation of estrogen therapy as a practical, clinical treatment for coronary atherosclerosis. The favorable blood lipid changes must be balanced against the undesirable effects of the hormone on the male patient. Whether the change in blood lipids slows or reverses the process of coronary atherosclerosis must await the findings of long-term studies of large, well-controlled series of patients. Until such time as there is evidence of benefit that outweighs the disadvantages of this treatment, it must be considered an experimental approach to the problem of coronary atherosclerosis.

Summary

Estrogens change the serum lipid patterns of survivors of myocardial infarction, causing a decrease in total cholesterol, an increase in phospholipid, and an increase in \( \alpha \)-lipoprotein cholesterol. Consequently, the C/P ratio and the \( \beta/\alpha \)-lipoprotein cholesterol ratio fall. In general, these changes were seen as early as 1 month, were more pronounced at 3 months, and were maintained thereafter.

Total urinary 17-ketosteroid excretion was not significantly depressed by estrogens; 11-oxygenated ketosteroids were not decreased. Androsterone and etiocholanolone were substantially decreased, presumably due to decreased testicular function. There was a consistent rise in dehydroisoandrosterone excretion.

Sexual potency gradually decreased and became absent on 10 mg. of Premarin daily. Testicular biopsies showed fibrosis of tubular cells and severe atrophy or complete absence of Leydig cells at 1 year.

Thyroid function, as measured by I uptake, was not altered by 10 mg. of Premarin daily for 1 year. The observed serum lipid changes could not, therefore, be ascribed to increased thyroid function.

Estrogen therapy of coronary atherosclerosis should still be regarded as an experimental approach to this problem.

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Summary in Interlingua

Estrogenos altera le configurationes del lipidos seral in superviventes de infarimento myocardial: Illos causar un reduction del cholesterol total, un augmento de phospholipido, e un augmento de cholesterol a lipoproteina alpha. Il occurre per consequente un reduction del proportion C:P e del proportion beta:alpha de cholesterol a lipoproteina. In general iste alterationes esseva notate post non plus que un mense. Illos esseva plus pronunciata post 3 menses. Postea illos remaneva constante.

Le total excretion urinari de 17-ketosteroides non esseva significativamente deprimite per estrogenos. Le cetosteroides 11-oxygenate non
eseva redutte. Androsterona e etiocholanolona eseva redutte a grados considerabile, probabilmente in consequentia de deprimite functiones testicular. Il habeva un augmento regular in le excretion de dishydrostroandosterone.

Le potentia sexual decresceva gradualmente e dispareva con 10 mg de Premarina per die. Biopsias testicular monstrava fibrosis de cellulas tubular e sever atrophia o complete absentia de cellulas de Leydig post 1 anno.

Le functiones thyroide, mesurate per le acceptation de $I^{31}$, non eseva alterate per 10 mg de Premarina per die durante 1 anno. Le observe alterationes de lipidio seral eseva assi non ascribibile a augmentos de function thyroide.

Le therapia a estrogeno in atherosclerosis coronari debe ancora esser considerate como un procedimento experimental.

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