Effect of Estrogens on Interlipid Relations in Men with Myocardial Infarction.* (28365)

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The rather close correlation between serum cholesterol and phospholipids suggests that they are independent variables which tend to rise or fall together in some systematic manner. Popjak(1) examined this interlipid relationship using bivariate regression methods and found that the log-log linear regression of phospholipids on cholesterol appeared to be quantitatively constant in a number of species, ages and clinical states (although he did not especially consider the situation in atherosclerosis).

We have recently confirmed his general observation in men but noted that those with clinical manifestations of atherosclerosis (myocardial infarction or cerebral thrombosis) showed a distinct and significant abnormality in their interlipid relationships (2). This abnormality consisted of a lower-than-normal level of phospholipid associated with a given level of cholesterol. This finding, we believe, sheds new light on the well known finding that men with coronary disease tend to average a somewhat higher level of cholesterol and a higher cholesterol/phospholipid (C/P) ratio than normal(3-7). This difference in averages is rather small, amounting to some 10%, and cholesterol level has little value in predicting the occurrence of new complications(8). Nonetheless, cholesterol has been so thoroughly incriminated in atherogenesis that any agent which lowers serum cholesterol or C/P ratio tends *ipso facto* to be viewed as antiatherogenic.

One such agent is estrogen, whose ability to lower serum cholesterol and/or raise phospholipids has been amply demonstrated(9-19). More direct evidence also exists that estrogens may actually be antiatherogenic—as the rarity of myocardial infarction in cyclic women, experimental demonstration of antiatherogenesis in animals(20) and autopsy findings in men given estrogen in treatment of cancer(21). We are concerned here, however, with the effect of estrogens upon the serum lipids.

Our findings indicate that treatment for 6 months with Premarin (mixed conjugated equine estrogens, NNR) failed to change the abnormality in interlipid relationships characteristic of men with myocardial infarction while treatment with Lynoral (ethinyl estradiol) abolished this abnormality. Results with Anvene (mytatrienediol, SC 6924: 3 methoxy-16-methyl-1,3,5(10)-estratriene-16β,17β-diol) were equivocal although it did effect a significant reduction in mean cholesterol. All 3 estrogens were employed in doses causing minor breast changes and were therefore roughly comparable in feminizing potency. As noted elsewhere(22,23), Premarin significantly improved survival rate and reduced the incidence of new infarctions while Lynoral and Anvene did not.

Material and methods. Clinical material, drawn from Los Angeles County Hospital

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and Cedars of Lebanon Hospital, consisted of men who had recovered from a frank myocardial infarction, who were free of concomitant illness deemed a threat to life, who were cooperative and able to attend special atherosclerosis research clinics regularly and who were willing to accept estrogen therapy if prescribed. After complete clinical studies by physicians regularly employed in these clinics, patients accepted for study were allocated by the statistical control office, using randomized methods, to one of 4 treatment groups: No estrogen (No), Premarin (Pr), Lynoral (Ly) or Anvene (An). Dosage protocol called for commencing with small doses and increasing these to the point of minor clinical manifestation, such as breast tenderness. Dosage thereafter was continued at a level well tolerated by the individual (usually .05-.15 mg Lynoral, 1.25-2.5 mg Premarin or 10-15 mg Anvene daily by mouth).

Serum cholesterol was determined by the method of Pearson(24) and phospholipids by the procedure of Lowry(25). Although many lipid determinations were made, we have reported(18,19) that lipid changes tended in this clinical trial to become maximal in about 3 months. We have therefore selected for our present purpose the last determination made before start of treatment (I) and the first determination made following 6 months of continuous treatment (II), using all available data from the clinical trial in which these conditions were met.

For reasons indicated elsewhere(1,2), all data were transformed to their logarithms to the base 10. Linear regressions were calculated by the method of least squares, and t tests of the significance of differences in their coefficients were calculated in the usual way (26). Multiple comparisons among group means were evaluated by the Dunn multiple t test(27). P values of .05 or less were considered significant.

Results. The salient statistics are shown in Table I. Before the start of treatment (I), the 4 treatment groups did not differ significantly in mean cholesterol, mean phospholipids, in regression coefficients, b, in standard deviations of cholesterol or of phospholipids, or in values of P′ calculated for various levels of C from the regression equation Log P′ = a + b Log C, P and C being phospholipids and cholesterol, respectively. Fig. 1 shows the log-log linear regressions of the 4 treatment groups in interval I, before start of treatment, using arithmetical ordinates.

In the No estrogen and Premarin groups there was no appreciable change in mean cholesterol or mean phospholipids between intervals I and II. In the Lynoral group, mean cholesterol fell about 8% from 249 to 230 ($p = .05$) while phospholipids rose about 8% from 233 to 253 mg % ($p = .02$). In the Anvene group, the 10% fall in cholesterol, from 258 to 233 mg %, barely achieved significance at the .01 level while mean phospholipids remained essentially unchanged. There was no significant change in standard deviations of cholesterol or phospholipids under treatment. Though the dif-

TABLE I. Summary of Essential Data for Each Treatment Group: No: No estrogen; Pr: Premarin; Ly: Lynoral; An: Anvene; n: No. of cases; C, P, V_C and V_P: means and variances of log cholesterol and log phospholipids; a and b: constants of regression equation, Log P′ = a + b Log C; V_b: variance of b; and r: coefficient of correlation between Log P and Log C. Interval I is the last lipid determination prior to start of treatment and II the first following 6 months of continuous treatment.

Group	n	C	P	V_C	V_P	a	b	V_b	r
I: Before treatment:									
NoI	72	2.3930	2.3454	.007230	.006890	.5969	.7307	.005986	.75
PrI	41	2.3921	2.3464	.007250	.008440	.2891	.8600	.010886	.80
LyI	65	2.3958	2.3681	.007970	.006560	.8370	.6390	.006583	.70
AnI	45	2.4117	2.3733	.005030	.005790	1.2455	.4677	.021684	.44
II: After treatment									
NoII	72	2.3797	2.3466	.009410	.005580	1.3109	.4352	.005766	.57
PrII	41	2.3882	2.3400	.006090	.007940	.2047	.8941	.012931	.78
LyII	65	2.3617	2.4030	.007630	.005530	1.4560	.4010	.008951	.47
AnII	45	2.3674	2.3802	.007590	.012420	1.8207	.2363	.036756	.18

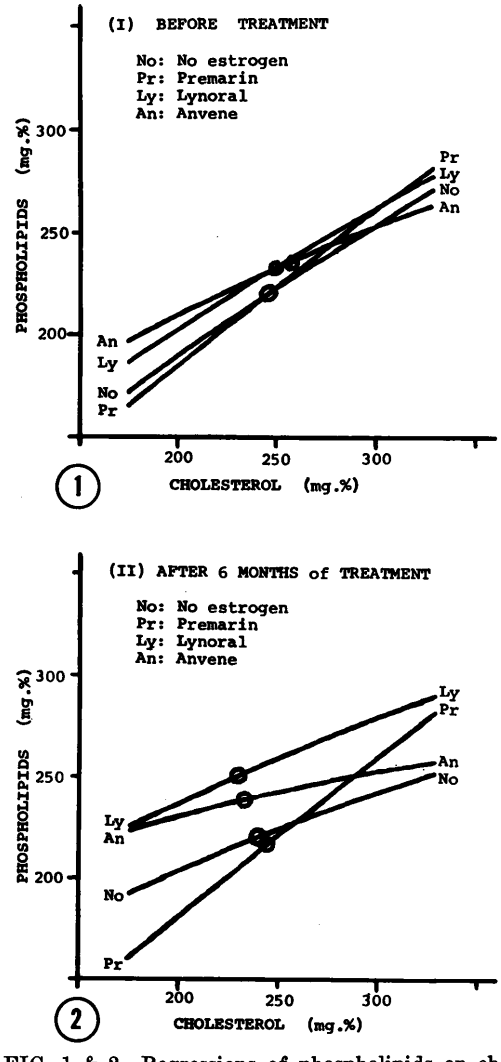

FIG. 1 & 2. Regressions of phospholipids on cholesterol in the four treatment groups. Fig. 1, before therapy and Fig. 2, after 6 mo of therapy. Means are denoted by circles.

ferences lacked significance, there tended to be a reduction in degree of correlation between cholesterol and phospholipids in interval II, as compared with interval I, in the No estrogen, Lynoral and Anvene groups, these reduced correlations being reflected in the smaller b coefficients in interval II in these groups.

In interval II, the b coefficients of the No estrogen, Lynoral and Anvene groups all differ significantly from that of the Premarin group, as shown in Fig. 2, which exhibits the regressions in the 4 groups after 6 months of treatment. It is not clear whether these changes in b coefficients are incidental statistical artifacts resulting largely from nonsignificant changes in degree of correlation or are real phenomena requiring further exploration and interpretation. The former is suggested by the otherwise observed uniformity of these coefficients(1,2). In any event, the question remains of whether the curves of Fig. 2 are significantly *higher* than those in Fig. 1: *i.e.*, whether the interlipid abnormality in men with atherosclerosis has been abolished by treatment with these estrogens in the doses used.

The elevations of 2 regressions may be compared by calculating the level of the nominally dependent variable (P′) for a selected value of the nominally independent variable (C). If the 2 sample regressions happen to have identical slopes, then this comparison may be made at any desired point which seems germane to the inquiry, or if there is no logical basis for selecting some particular value of C for this purpose, one may arbitrarily select any value and learn whether it yields a significant difference. When the 2 regressions differ in slope, however, the calculated values of P′ depend for their differences, not only on elevation but also on slope, the contribution of the latter increasing with distance from the mean. In this series, the 8 cholesterol means (4 in I and 4 in II) range from 230 to 258 mg %. Since we are reporting elsewhere(2) the values of P′ calculated for cholesterol levels of 225 and 255 mg %, and since the contribution of differences in b to differences in calculated P′ levels is trivial within this small range from the observed means, we have arbitrarily elected to utilize these same 2 cholesterol levels in the present analysis. The level of phospholipid (P′) calculated from the various regressions for these 2 selected levels of cholesterol (C) are shown in Table II before (I) and after 6 months of treatment (II). Table III shows probability levels, where significant, for the differences between these calculated phospholipid levels.

There was no significant difference among the 4 treatment groups before the start of therapy. Under treatment with Lynoral there was a significant increase in phospholipids, not only in comparison with their

TABLE II. Levels of Phospholipids (P') Calculated from the Various Regression Equations for Cholesterol (C) Levels of 225 and 255 mg %. Data for normal males (WC) reported elsewhere(2).

Group		P' calculated for: C = 225	C = 255
Normal males	WC	226	246
Present series: I: Before treatment:			
No estrogen	NoI	207	227
Premarin	PrI	205	229
Lynoral	LyI	219	237
Anvene	AnI	222	235
Present series: II: After treatment:			
No estrogen	NoII	216	228
Premarin	PrII	203	228
Lynoral	LyII	251	264
Anvene	AnII	238	245

previous level in these same persons but also in comparison with the other treatment groups. Phospholipids also tended to rise in the Anvene group under therapy, though here the differences were less marked and failed to achieve significance. The groups receiving Premarin or No estrogen showed no appreciable change during treatment.

The previously reported abnormality in interlipid relationships characteristic of clinical atherosclerosis persisted unchanged during 6 months of treatment with Premarin or No estrogen but was abolished by treatment with Lynoral(2). The effects of Anvene treatment upon these interlipid relationships were uncertain although, as noted above, there was a significant reduction in mean cholesterol in this group.

Discussion. We have noted elsewhere that men with clinical manifestations of atherosclerosis (myocardial infarction or cerebral thrombosis) show a significant abnormality in interlipid relationships, characterized by a lower level of phospholipid associated with any given level of cholesterol. We also observed, in confirmation of Popjak(1), that in various other clinical states, which may show marked differences in both mean cholesterol and mean phospholipid, the interlipid relationship tends to remain quantitatively constant. We believe this observation to be clinically important because it indicates some basic abnormality in atherosclerosis that is present over a broad spectrum of lipid levels.

Data presented here indicate that treatment with Lynoral actually resulted in abolishing this abnormality. In men receiving Premarin, and in those treated with No estrogen, there was no significant change in interlipid relationships. It should be emphasized that treatment utilized small, well-tolerated doses causing minor breast manifestations. In the case of Premarin, doses were usually 1.25 to 2.5 mg/day. While other investigators have not reported the effect of Premarin on interlipid relationships, they have noted that little or no change in mean cholesterol or phospholipid occurs in men given small doses of this preparation(28).

The positive effect of Lynoral on the serum lipids and the lack of any effect of Premarin, in the doses used in this study, are of great interest in conjunction with their effects, in this same clinical trial, on (a) production of clinical manifestations(29) of estrogen therapy (such as breast changes) and (b) incidence of death or new events (22,23). All 3 estrogenic preparations were given in dosages causing minor breast changes that were easily tolerated by the individual subject: thus all 3 estrogens were given in approximately equal doses as gauged by "feminizing" potency. Lynoral and Anvene failed to improve survival rate or reduce the frequency of new events, but significantly altered the serum lipids toward those found in normal individuals. Premarin, on the other hand, appears to improve survival rate with no effect on the serum lipids.

We therefore have observed dissociation among 3 effects of estrogens given to men with myocardial infarction: the estrogen effect, serum lipid effect and clinical outcome effect. In each of these respects, Lynoral and Anvene appear quite similar; both of

TABLE III. Probability Values for Comparisons Found Significant by the Dunn Multiple Comparisons Test(27) for Differences Between P' Levels Shown in Table II. Critical t ratios used are 3.30 for $P < .05$ and 3.80 for $P < .01$.

	P'_C=225	Probability	P'_C=255	Probability
NoII – LyII	216-251	.01	228-264	.01
PrII – LyII	203-251	.01	228-264	.01
PrII – AnII	203-238	.05	228-245	—
LyI – LyII	219-251	.01	237-264	.05

these preparations are synthetic, chemically pure estrogens. Premarin, however, is a natural preparation containing estrogenic as well as possibly non-estrogenic components. Our findings suggest that it may contain constituents relatively potent in influencing survival in men with myocardial infarction without modifying serum lipids. These data suggest that reduction of cholesterol or C/P ratio is not necessarily clinically beneficial and substances that are antiatherogenic need not necessarily modify the serum lipids.

Conclusions. 1. A total of 223 men recovered from myocardial infarction were allocated to treatment groups and serum cholesterol and phospholipids determined (I) before and (II) following 6 months of uninterrupted treatment with No estrogen, Premarin, Lynoral or Anvene. 2. The abnormality in interlipid relationships characteristic of men with clinical atherosclerosis was unaltered in the subjects receiving Premarin, but this abnormality was abolished by treatment with Lynoral. 3. There is no necessary correlation between the feminizing, lipid-altering and outcome-altering effects of estrogen therapy in men with myocardial infarction.

1. Popjak, G., *Biochem. J.,* 1946, v40, 608.

2. Moore, F. J., Marmorston, J., Kuzma, O. T., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 350.

3. Gertler, M. M., White, P. D., *Coronary heart disease in young adults.* Havard Univ. Press, Cambridge, 1954.

4. Jolliffe, N., *New York State J. Med.,* 1957, v57, 2684.

5. Katz, L. N., Stamler, J., Pick, R., *Nutrition and Atherosclerosis,* Lea and Febiger, Philadelphia, 1958.

6. Mattill, H. I., *Cholesterol as Related to Atherosclerosis.* Cereal Institute, Inc., Chicago, 1959.

7. Pincus, G., Ed., *Hormones and Atherosclerosis.* Academic Press, Inc., New York, 1959.

8. Goffman, J. W., *et al., Circulation,* 1956, v14, 691.

9. Eilert, M. L., *Am. Heart J.,* 1949, v38, 472.

10. ———, *Metabolism,* 1953, v2, 137.

11. Russ, E. M., Eder, H. A., Barr, D. P., *Am. J. Med.,* 1955, v19, 4.

12. Steiner, A., Payson, H., Kendall, F. E., *Circulation,* 1955, v11, 784.

13. Oliver, M. F., Boyd, G. S., *ibid.,* 1956, v13, 82.

14. Robinson, R. W., Higano, N., Cohen, W. D., Sniffen, R. C., Sherer, J. W., Jr., *ibid.,* 1956, v14, 365.

15. Furman, R. H., Howard, R. P., Norcia, L. N., Keaty, E. C., *Am. J. Med.,* 1958, v24, 80.

16. Cohen, W. D., Higano, N., Robinson, R. W., *Circulation,* 1958, v17, 1035.

17. Marmorston, J., Magidson, O., Lewis, J. J., Mehl, J., Moore, F. J., Bernstein, J., *New England J. Med.,* 1958, v258, 583.

18. Marmorston, J., Moore, F. J., Magidson, O., Kuzma, O., Lewis, J. J., *Ann. Int. Med.* 1959, v51, 972.

19. Marmorston, J., Moore, F. J., Lewis, J. J., Magidson, O., Kuzma, O., *Clin. Pharm. and Therap.,* 1960, v4, 449.

20. Stamler, J., Pick, R., Katz, L. N., *Ann. N. Y. Acad. Sci.,* 1956, v64, 596.

21. Rivin, A. U., Dimitrcff, S. P., *Circulation,* 1954, v9, 533.

22. Marmorston, J., Moore, F. J., Kuzma, O. T., Magidson, O., Weiner, J., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 618.

23. Marmorston, J., Moore, F. J., Hopkins, C. E., Kuzma, O. T., Weiner, J., *ibid.,* 1962, v110, 400.

24. Pearson, S., Stern, S., McGavack, T. H., *Analyt. Chem.,* 1953, v25, 813.

25. Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., Farr, A. L., *J. Biol. Chem.,* 1954, v207, 1.

26. Moore, F. J., Cramer, F. B., Knowles, R. G., *Statistics for Medical Students.* Blakiston, Philadelphia, 1951.

27. Dunn, O. J., *J. Am. Statist. Assn.,* 1961, v56, 52.

28. Stamler, J., Pick, R., Katz, L. N., Pick, A., Kaplan, B. M., *Hormones and Atherosclerosis,* G. Pincus, Ed., Academic Press, Inc., New York, 1959, p423.

29. Marmorston, J., Magidson, O., Kuzma, O., Moore, F. J., *J.A.M.A.,* 1960, v174, 241.

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