Quantitative Study of Influence of Estrogenic Substances on Serum Lipids of Rats Fed Atherogenic Diet.* (23460)

R. E. PRIEST, M. T. SCHROEDER, R. RASMUSSEN, AND R. W. WISSLER
Department of Pathology, University of Chicago

The relative infrequency with which clinical manifestations of coronary atherosclerosis are encountered in human females during the reproductive years is well known(1,2). This observation constitutes an unusual example of a natural protective metabolic mechanism which is capable of offsetting atherogenic dietary influences. Clinical observations(3,4) and experimental work with chickens(5,6) and rats(7) have suggested that this protection is related to the effects of estrogens, but the use of estrogens in the treatment of coronary atherosclerosis has been hampered by the feminization which occurs in male patients. Although the mechanisms by which estrogens influence atherosclerosis are not fully understood, clinical(8,9,10) as well as experimental(5,7) studies suggest that suppression of atherosclerosis by estrogens is associated with a higher serum level of phospholipids relative to cholesterol. Since the ratio of cholesterol to phospholipids appears to be associated with development of atherosclerosis in several species under widely diverse circumstances(5,7,11-15) this effect of estrogen on blood lipids may be of primary importance.

The purpose of the present study was 2-fold: first, to obtain additional quantitative information regarding effects of estrogenic substances on serum lipids, and second to see if substances chemically related to estrogens could be demonstrated to influence the serum lipids without causing undesirable changes in the male reproductive organs.

Materials and methods. Animals. In all experiments adult male albino rats weighing between 400-500 g were divided into groups of similar weights and ages. *Diet.* A liquid ration as described by Moskowitz(7) containing 2% protein, 25% fat in the form of corn oil and 2% cholesterol was administered by gastric tube 3 times each day to each animal. During the first week of experimentation the amount of diet administered was gradually increased from 10 ml per feeding up to 12 or 13 ml. The animals were allowed tap water *ad lib.*, but no additional diet. *Estrogen and estrogen analogues.* After the first week of the experiment each animal was given daily (excepting Sundays), subcutaneous injections of the substances dissolved or suspended in sesame oil. *Serum lipids.* Fasting blood samples were obtained from a lateral tail vein of each animal at the beginning of an experiment and at weekly intervals thereafter. For each group equal amounts of blood from each animal were pooled, the serum obtained by centrifugation and total cholesterol and lipid phosphorus determined by standard methods (16,17).

Results. When differing amounts of estradiol benzoate were given, differing effects were obtained (Fig. 1). Among the estradiol-treated animals the largest amount given produced the least elevation of serum cholesterol, but the greatest elevation of phospholipid. Conversely, the least amount of estrogen produced the greatest rise in cholesterol and the least rise in phospholipids. The intermediate amount of estradiol caused an intermediate elevation of both cholesterol and phospholipid. Consequently, the animals which received the smallest amount of estrogen had greatly elevated C/P ratios, while those that received the largest amount had C/P ratios as low or lower than the controls. At all levels of estradiol atrophy of testes, prostate and seminal vesicles occurred.

It should be emphasized that the animals used in these experiments were adult males. The interplay between androgens and estrogens in regard to effects on serum lipids is yet to be fully worked out, but from these results it can be concluded that, at least with adult male rats, one is not justified in speaking of "estrogen effects." The character of the effects depends to a large extent upon how

* This investigation was supported by grants from the Chicago Heart Assn. and from the Nat. Heart Inst. N.I.H., U. S. Public Health Service.

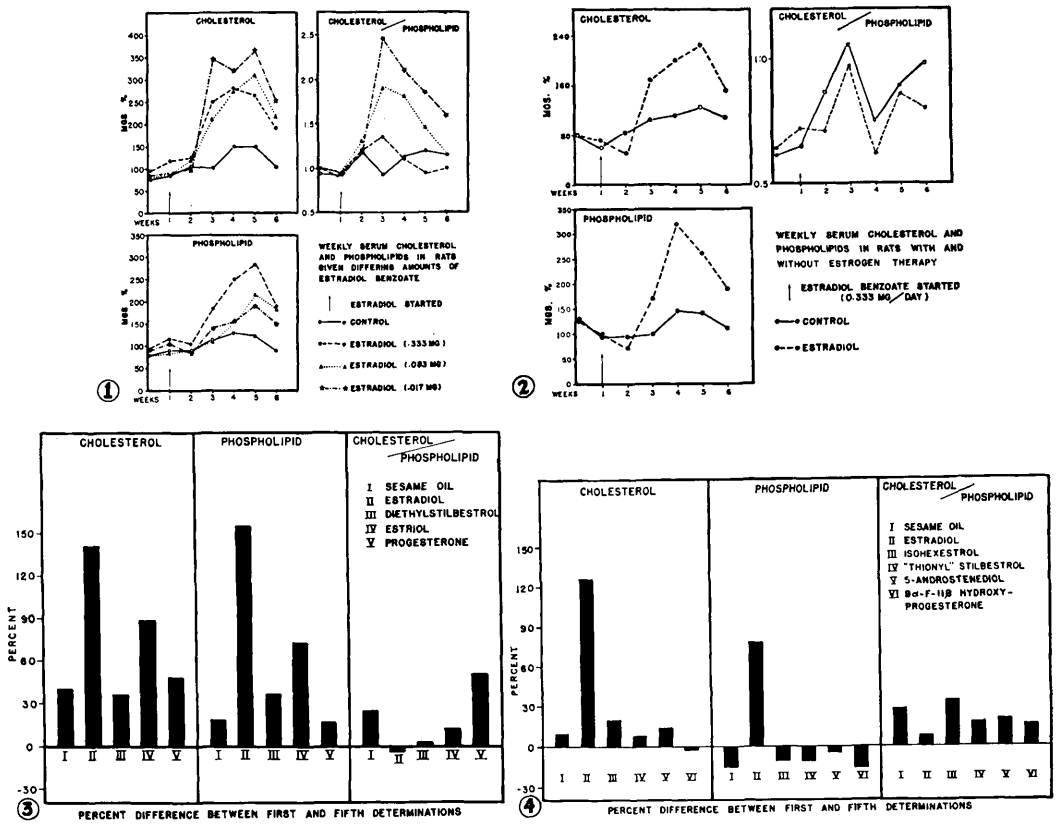

FIG. 1-4.

much estrogen is administered. Because daily administration of 0.333 mg estradiol produced the lowest C/P ratios, this amount was chosen as a reference in the subsequent experiments.

Consequently, in each of the experiments reported here appropriate control groups, one receiving 0.333 mg estradiol benzoate daily and the other receiving only sesame oil were included. When these two groups were compared, the same general pattern of changes in serum lipids with time as that depicted in Fig. 2 was observed in 3 additional experiments. At the beginning of the experiment values for cholesterol, phospholipid and C/P were about the same for treated and control groups. This was a constant observation in these experiments. Both cholesterol and phospholipid levels fell slightly at the end of the first week. By the end of the third week both were elevated, but this elevation was much more marked in the animals which had received estradiol. After reaching a peak a decrease in both cholesterol and phospholipids occurred. The C/P ratio for the group receiving estradiol was lower than that of the control group during the latter part of these 6-week experiments.

With these quantitative relationships in mind a number of natural and synthetic female hormones as well as structurally related compounds were compared. The promotion of the rat's uterine growth by many of these substances has been studied in detail by Huggins and Jensen† (18,19).

Two experiments were designed as shown in Table I.

When the percent difference between values for serum lipids at the end of the fourth week and at the beginning of the experiment were compared (Fig. 3 and 4), both cholesterol and phospholipids were found to have been most

† Some of these compounds were given to us by Dr. Elwood V. Jensen.

TABLE I. Kinds and Amounts of Hormones and Analogues Administered Daily in 2 Experiments. Five animals per series.

Group	"Hormone"	Molecular wt	Amt (mg/day)
I	Control		
II	Estradiol benzoate	271	.333
III	Diethylstilbestrol	268	"
IV	Estriol	287	"
V	Progesterone	314	"
I	Control		
II	Estradiol benzoate	271	.333
III	Isohexestrol	270	"
IV	"Thionyl stilbestrol"*	258	.317
V	5 androstene 3B 17B diol	291	.358
VI	9X fluoro 11B hydroxy-progesterone	351	.433

* 3-(2 thienyl)-4-(e-hydroxy-phenyl)-hexene-3.

elevated in the groups which received estradiol and estriol. The phospholipids were proportionately more elevated than the cholesterol in the group given estradiol, resulting in a fairly constant C/P ratio. Administration of stilbestrol also resulted in maintenance of a fairly constant C/P ratio. To this extent its effect was like estradiol, since the values for the control group rose during this period. Progesterone gave results more like the control group, as did all of the analogues tested.

As can be seen from Table II, the 2 natural estrogens, as well as stilbestrol and isohexestrol caused atrophy of the reproductive glands to about the same degree, while the other estrogen analogues produced atrophy to a lesser degree. Although progesterone apparently produced atrophy in this experiment it did not do so in another.

Atrophy of the male sexual organs occurred with all of the substances studied which had an effect on serum lipids similar to that of estradiol.

Discussion. The reported effects of estrogenic substances on blood lipid concentrations and on atherogenesis are remarkably variable. For example with 1 mg per day of estradiol benzoate for 13 weeks, Pick *et al.*(5) reported suppression of coronary atheromatous lesions in the chicken accompanied by a definite rise in blood lipid phosphorus and reversal of coronary lesions with 1 mg per day for 5 weeks (6). Other workers(20,21) have indicated an increase in atherogenesis and hyperlipemia in this same species when 25 mg pellets of the somewhat less potent diethylstilbestrol were implanted about once a month for varying periods. In the estrogen-treated rat, Moskowitz *et al.*(7) observed a marked rise in both serum cholesterol and lipid phosphorus as compared to controls when the animals were force-fed a high fat diet. In this study 0.333 mg of estradiol per day appeared first to lead to increased susceptibility to coronary atheromatous lesions followed later by suppression or reversal of the lesions. Other results in the rat have been reported recently by Fillios *et al.*(22) who found that 0.02 mg of estradiol injected 2 times per week for 7 weeks increased the serum cholesterol concentration. Under similar experimental conditions estrogen treatment enhanced cardiovascular sudanophilia has been reported in male rats (23).

The results of the present study suggest that at least some of this variation in the effects of estrogenic substances may be related to the dose and period of administration. Similarly the biphasic effect on atherogenesis produced by estradiol in the rat(7) may be explained by a gradually increasing daily effect of the estrogen resulting from multiple sesame oil depots which may superimpose their liberated estradiol.

It will be necessary to find out whether the differing effects of various doses of estrogen on blood lipids and atherogenesis are observed in other species. It is possible that this concept may have broad implications in terms of the natural history of atherogenesis in man. Observations relating estrogenic therapy to blood lipids in human(4,10) as well as in

TABLE II. Average Weights of Reproductive Glands of Rats Given Estrogens and Analogues.

Group	Prostate (g)	Testis (g) R	Testis (g) L
I	1.62	1.50	1.54
II	.73	.33	.36
III	1.01	.31	.32
IV	.97	.34	.35
V	1.03	.58	.60
I	2.69	1.65	1.59
II	1.52	.53	.53
III	1.61	.92	.72
IV	2.62	1.73	1.46
V	2.30	1.14	1.51
VI	1.92	1.34	1.36

other species(24) may need further evaluation in the light of dosage and time effects demonstrated in this study. The tendency for blood lipid values of the rat to decline after several weeks of therapy suggests that an adaptive mechanism may be present which will tend to counteract the early effects of estradiol even when a large dose is administered.

Recent observations(25) which relate lipid phosphorus rise in the human male to estrogenic analogue administration while demonstrating little evidence of estrogenic effect on secondary sex characteristics represent a desirable goal and deserve further evaluation in experimental animals as well as in man.

Summary. 1) In adult male albino rats varying amounts of estradiol, all within a range sufficient to produce testicular atrophy, differed in their effects on serum lipids, the smaller amounts causing marked elevation in serum cholesterol and less elevation in phospholipids than larger amounts. Administration of 0.333 mg estradiol benzoate daily resulted in marked elevation of serum cholesterol and phospholipids within 2 weeks, but the C/P ratio remained as low or lower than that of control animals. Over a 5-week period these values reached a peak, then fell in spite of constant diet and hormonal treatment. 3) Equivalent amounts of estriol daily had an effect on serum lipids similar to that of estradiol. 4) Equimolecular amounts of stilbestrol, progesterone, and a variety of substances chemically related to active estrogens had little acute effect on serum lipids under the conditions studied. 5) This experimental plan may be useful for studying simultaneous effects of other steroids on serum lipids and on the reproductive organs of mammals.

1. Gover, M., and Pennel, M. Y., *Pub. Health Rep.,* 1950, v65, 819.

2. Levy, R. L., Bruenn, H. G., and Kurtz, D., *Am. J. Med. Sci.,* 1934, v187, 376.

3. Wuest, J., Dry, T. J., and Edwards, J. E., *Circulation,* 1953, v7, 801.

4. Rivin, A. U., and Dimitroff, S. P., *ibid.,* 1954, v9, 533.

5. Pick, R., Stamler, J., Rodbard, S., and Katz, L. N., *ibid.,* 1952, v6, 276.

6. ———, *ibid.,* 1952, v6, 858.

7. Moskowitz, M. S., Moskowitz, A. A., Bradford, W. L., and Wissler, R. W., *Arch. Path.,* 1956, v61, 245.

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

9. Eilert, M. L., *Metabolism,* 1953, v2, 137.

10. Barr, D. P., *J. Chronic Diseases,* 1953, v1, 63.

11. Gertler, M. M., Garn, S. M., and Lerman, J., *Circulation,* 1950, v2, 205.

12. Kellner, A., Correll, J. W., and Ladd, A. T., *Am. Heart J.,* 1949, v38, 460.

13. Wissler, R. W., Eilert, M. L., Schroeder, M. A., and Cohen, L., *Arch. Path.,* 1954, v57, 333.

14. Steiner, A., Davidson, J. D., and Kendall, F. E., *Am. Heart J.,* 1948, v36, 477.

15. Mann, G. V., Andrus, S. B., McNally, A., and Stare, F. J., *J. Exp. Med.,* 1953, v98, 195.

16. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

17. Fister, H. J., *Lipid Phosphorus, Method* P-37.1, *Manual of Standardized Procedures for Spectrophotometric Chemistry,* N. Y. Standard Scientific Supply Corp. 1950.

18. Huggins, C., and Jensen, E. V., *J. Exp. Med.,* 1955, v102, 335.

19. ———, *ibid.,* 1955, v102, 347.

20. Chaikoff, I. L., Lindsay, S., Lorenz, F. W., and Entenman, C., *J. Exp. Med.,* 1948, v88, 373.

21. Lindsay, S., and Chaikoff, I. L., *Arch. Path.,* 1950, v49, 434.

22. Fillios, L. C., *Endocrinology,* 1957, v60, 22.

23. Fillios, L. C., and Andrus, S. B., *Fed. Proc.,* 1957, v16, 356.

24. Fillios, L. C., and Mann, G. V., *Circulation Res.,* 1956, v4, 406.

25. Robinson, R. W., Cohen, W. D., and Higano, N., *Circulation,* 1956, v14, 489.

Received June 13, 1957. P.S.E.B.M., 1957, v96.