The Influence of Estradiol on the Distribution of Exogenous Cholesterol-4-C^{14} in the Rat

Several possible mechanisms of estrogen action in the atherogenic process have been postulated. It has been shown that estrogens may act directly on the arterial wall to influence the enzymes involved in aerobic metabolism[1,2] and mucopolysaccharide synthesis[3,4]. Other studies have indicated that estrogens may stimulate the reticulo-endothelial system and thereby increase the metabolism of cholesterol[5-7]. Several in vitro investigations have demonstrated that cholesterol oxidation by liver mitochondria may be enhanced by estrogens[8,9].

The aims of the present experiments were to study the immediate effects of estradiol on the retention of exogenous cholesterol, so as to further elucidate the mechanisms of estrogen action in lipid metabolism and atherosclerosis.

Thirty-two male three-month-old Sprague-Dawley rats on a commercial diet (Purina) were divided into four groups of eight each. Groups III and IV were orchiectomized and allowed two weeks for recovery. All rats were then placed in individual metabolism cages and received daily for 10 consecutive days subcutaneous injections of the following: Group I, 33 μg estradiol benzoate (Progynon) in 0.3 ml sterile sesame oil; Group II, 0.3 ml sterile sesame oil; Group III, 33 μg estradiol benzoate in 0.3 ml sesame oil; Group IV, 0.3 ml sesame oil. On the fifth day of injections, each rat received by stomach tube 7.0 μC of cholesterol-4-C^{14} (Nuclear-Chicago, Specific Activity 20 mC/millimole) per 100 g body weight. Feces were collected daily from each rat and stored at -20 °C. After the tenth day the rats were killed, portions of hearts, aortas and livers sectioned and fixed, and autoradiographs prepared with Kodak NTB-2 nuclear track emulsion. The lipids of the livers, aortas, plasma and feces from each animal were extracted with methylal-methanol 4:1[10]. The residue from the fecal extracts was hydrolyzed with aqueous NaOH to free the bile acid conjugates, re-extracted with the methylal-methanol solution, and this extract added to the original. Aliquots of all the extracts were analyzed for total cholesterol[11], and the specific radioactivities of the hydrolyzed digitonin-precipitable and non-digitonin-precipitable substances determined in a PPO-POPOP scintillator using a Packard Model 3314 Liquid Scintillation Spectrometer. Quenching was monitored by the channels ratio technique, and all counts corrected to 100% efficiency with internal standards.

The probabilities that apparent differences in the data were due to chance was calculated by the t test.

Autoradiographs of the aorta showed in many areas a high density of radioactive cholesterol associated with the elastic lamellae; the concentration was greatest in those lamellae closest to the lumen. This pattern of distribution suggested that the cholesterol-containing lipoproteins entered the aorta by infiltration from the plasma, and that the elastic laminae retarded the passage of these lipoproteins through the arterial wall.

There was no detectable localization of radioactive cholesterol in any cell type or area of either the coronary arteries or the liver. Neither castration nor estrogen treatment seemed to alter the microscopic pattern of cholesterol distribution in the aorta, coronary arteries or liver. It is possible that cholesterol uptake by the reticulo-endothelial cells may be manifest only with excessive dietary intakes of cholesterol, or that cholesterol is metabolized by the reticulo-endothelial cells but does not accumulate there.

Various investigations of the effects of estrogens on serum lipids and lipid-containing vascular lesions in the rat[6,12-14] have produced differing results, which are to some extent explainable by the different estrogen preparations used, the presence or absence of excessive cholesterol in the diet, and the duration of the experiment. In one investigation, estradiol was found to prevent the appearance of spontaneous atherosclerotic lesions in intact male rats[13]. On the other hand, cholesterol-fed intact male rats given estradiol were observed by other investigators[14] to develop coronary sudanophilic lesions after 8 weeks of treatment. No lesions were found after 5 weeks of treatment, and after 20 weeks, the incidence of lesions was less

[1] M. R. Malinow, J. A. Moguilevsky, and E. Bumanshny, J. Atheroscler. Res. *1*, 128 (1961).
[2] M. R. Malinow and J. A. Moguilevsky, J. Atheroscler. Res. *1*, 417 (1961).
[3] H. Hilz and D. Uterman, Biochem. Z. *332*, 376 (1960).
[4] R. E. Priest and R. M. Koplitz, J. exp. Med. *116*, 563 (1962).
[5] J. H. Heller, R. M. Meier, R. A. Zucker, and G. W. Mast, Endocrinology *61*, 235 (1957).
[6] R. Pick, L. N. Katz, P. J. Johnson, and D. E. Century, Circulation Res. *11*, 811 (1962).
[7] G. S. Boyd, Fed. Proc. *11*, 86 (1962).
[8] D. Krichevsky, E. Staple, J. L. Rabinowitz, and M. W. Whitehouse, Am. J. Physiol. *200*, 519 (1961).
[9] D. Krichevsky, S. A. Tepper, E. Staple, and M. W. Whitehouse, J. Lipid Res. *4*, 188 (1963).
[10] R. J. Morin, S. Bernick, J. F. Mead, and R. B. Alfin-Slater, J. Lipid Res. *3*, 432 (1962).
[11] M. L. Nieft and H. J. Deuel jr, J. biol. Chem. *177*, 143 (1949).
[12] L. C. Fillios and S. B. Andrus, Fed. Proc. *16*, 356 (1957).
[13] M. R. Malinow and A. A. Peligrino, Am. Med. Ass. Arch. Path. *65*, 47 (1958).
[14] M. S. Moskowitz and R. W. Wissler, J. Atheroscler. Res. *1*, 423 (1961).

Distribution of ingested cholesterol-4-C^{14}[a]

Group No.	I	II	III	IV
Treatment	Intact	Intact + estrogen	Orchiectomized	Orchiectomized + estrogen
Plasma-TC	205 ± 10.1	186 ± 10.7	201 ± 12.2	189 ± 9.6
Aorta-TC	1.01 ± 0.09	0.94 ± 0.13	0.95 ± 0.12	0.58 ± 0.08
Liver-TC	778 ± 59.3	1030 ± 57.7	716 ± 42	949 ± 40.3
Feces-TS[b]	17,200 ± 961	19,400 ± 1133	16,900 ± 759	20,100 ± 867
Feces-NS[b]	10,500 ± 430	11,800 ± 331	11,400 ± 410	12,500 ± 369

[a] All figures are expressed as total disintegrations per min (specific activity × total amount of sterol or non-sterol in tissue) divided by 1000, with standard deviations. Abbreviations: TC = total cholesterol; TS = total sterols; NS = non-sterols (non-digitonin precipitable lipids). [b] Total of days 6-10.

than after 8 weeks, indicating an apparent biphasic effect of the estrogens. In the present study the orchiectomized rats treated with estrogens showed a lower retention of administered radioactive cholesterol in the aorta, even though the plasma radioactive cholesterol concentration was similar to that of the other groups (Table). Estrogen treatment in the intact group did not result in a lower aortic concentration of cholesterol, which may be the result of an antagonistic action of the circulating androgens. Both estrogen treated groups, however, showed an increased hepatic concentration of radioactive cholesterol, and an increased degradation and excretion of cholesterol, as indicated by the higher fecal content of both the digitonin-precipitable sterols and the non-digitonin-precipitable metabolites of cholesterol in these groups. Orchiectomy also seemed to increase the rate of degradation and excretion of the administered cholesterol. These results are consistent with the observations in other investigations that the oxidation of cholesterol by hepatic mitochondria in vitro is enhanced by prior estrogen treatment or castration of male rats[13]. It appears from the present studies that estradiol may effect a redistribution of cholesterol, possibly mobilizing it from the arteries and other tissues, and preferentially concentrating it in the hepatic pool, where it is metabolized in both the parenchymal and Kupfer cells and excreted in increased amounts into the intestine[15].

Zusammenfassung. Vorbehandlung mit Östradiolbenzoat führte bei kastrierten männlichen Ratten zu einer verminderten Aufnahme von oral zugeführtem Cholesterin-4-C^{14} in die Aorta. Bei kastrierten wie auch bei intakten Tieren war nach Östrogenbehandlung die Aufnahme von markiertem Cholesterin in die Leber erhöht. Durch Analyse der Faeces liess sich nachweisen, dass Östrogenbehandlung sowie Kastration die Sterolausscheidung beschleunigt.

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[15] This investigation was supported by a grant from the Los Angeles County Heart Association. Also supported in part by grants from the Attending Staff Association, Harbor General Hospital, and the U.S.P.H.S. General Research Support Grant awarded to U.C.L.A. The excellent technical assistance of MARTHA HSUI and B. SINOR is gratefully acknowledged.

Succinic Dehydrogenase and Isocitric Dehydrogenase Activities in Vitamin E-Sufficient and -Deficient Fetal Rats

Our recent study of the E-sufficient and -deficient rat fetuses[1] indicated increase of the acid phosphatase positive granules in the latter indicating liberation of lysosomes in the pathological condition caused by E-avitaminosis. Since lysosomal enzymes are known to affect the mitochondrial membranes[2], it was decided to study the activity of succinic dehydrogenase (SDH) which is a mitochondrial marker. The activity of isocitric dehydrogenase (IDH), another oxidative enzyme, was undertaken for comparison. Sections 45 μ in thickness from fresh frozen embryos of both types[3], 15-, 17-, 19- and 21-day-old (three of each age group) were cut. For SDH activity the method of BARKA and ANDERSON[4] with the addition of 1 ml of 0.5% NaCN, and for IDH activity that of DICULESCO et al.[5] were followed.

The results indicate the presence of SDH in the liver, the heart and the CNS in 15-day-old E-sufficient as well as E-deficient embryos. By the 17th day the enzyme was demonstrable in many other structures, viz. lung, skin, intestine, stomach, kidney, skeletal muscle, cardiac muscle, cartilage and mesentery (Figure 2). From the

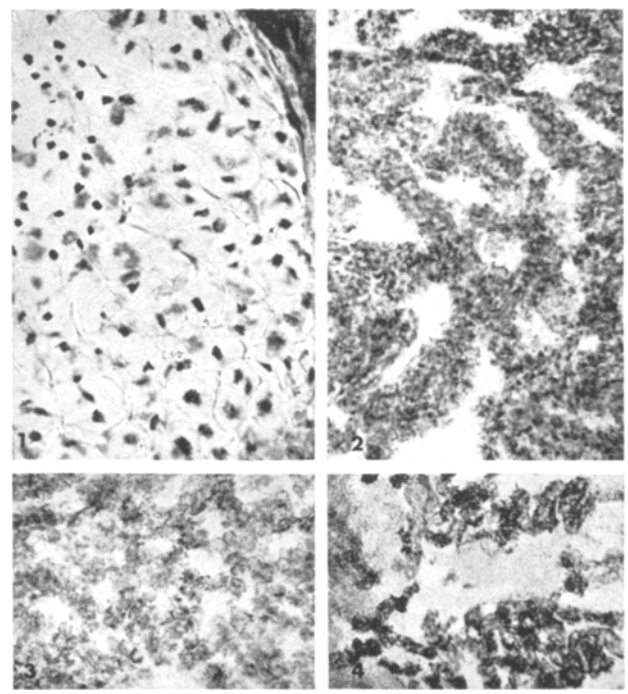

Fig. 1. Section of bone of a 21-day-old E-deficient fetus showing isocitric dehydrogenase activity in the different bone cell types. × 250.

Fig. 2. Section of the heart of a 17-day-old E-deficient fetus showing succinic dehydrogenase activity. × 240.

Fig. 3. Section of the liver of a 21-day-old E-deficient fetus showing succinic dehydrogenase activity. × 250.

Fig. 4. Section of the kidney of a 21-day-old E-deficient fetus showing succinic dehydrogenase activity. × 125.

[1] K. VERMA and D. W. KING, in press.

[2] A. L. TAPPEL, P. L. SAWANT, and S. SHIBKO, in *Lysosomes* (Eds. A. V. S. DE REUCK and M. P. CAMERON; Little, Brown and Co., Boston 1963).

[3] D. W. CHENG, L. F. CHANG, and T. A. BAIRNSON, Anat. Rec. *129*, 167 (1957).

[4] T. BARKA and P. ANDERSON, *Histochemistry* (Harper and Row, Inc., New York 1963).

[5] I. DICULESCO, D. ONICESCO, and L. MISCHIU, J. Histochem. Cytochem. *12*, 145 (1964).