to zero 2 to 3 weeks before weight losses began. At the same time, the rhodopsin level declined and the light threshold for the electroretinogram began to increase. The first significant change in QSO_4 rejection by our rats occurred in the third week preceding weight losses, which would correspond to the time when the supply of vit. A was completely depleted. However, since thresholds were not measured, changes may have occurred earlier. The late appearance of changes in NaCl selection and the lack of recovery of QSO_4 rejection suggests the possibility of different mechanisms underlying gustatory stimulation.

The rapid recovery of NaCl selection tends to rule out peripheral nerve damage or physical blocking of the taste pore as the reason for the taste deficit. A direct effect on the taste cells may reasonably be hypothesized. Vit. A may thus play a role in the functional and/or structural integrity of the taste cells. Verification will require histological and electrophysiological studies.

Further, the effect of vit. A may be specific or merely the result of a general effect on epithelial tissue. Dowling and Wald(4) reported that vit. A acid maintained normal growth in rats but did not prevent development of night blindness, thus limiting the role of vit. A to the visual system. An experiment is now underway in our laboratory to determine whether vit. A acid may also fail to prevent development of a taste deficit. If this is the case, vit. A would be implicated as a specific requirement in taste as in vision.

Summary. Rats in the advanced stages of vit. A depletion showed abnormal taste responses. A marked decrease in rejection of quinine and selection of NaCl solutions occurred. Administration of vit. A resulted in rapid recovery of NaCl selection but not of quinine rejection. These data suggest that vit. A may have a direct effect on the functioning of the taste cells.

1. Seifter, E., Rothberg, H., Berkman, J. I., *Abst. of Papers*, 1960, Am. Chem. Soc. 137th Meeting, 17N.

2. Siegel, S., *Non-Parametric Statistics*, 1956, McGraw Hill, New York.

3. Dowling, J. E., Wald, G., *Proc. Nat. Acad. Sci. U. S.*, 1958, v44, 648.

4. ————, Wald, G., *ibid.*, 1960, v46, 587.

Received September 22, 1961 P.S.E.B.M., 1961, v108

Effect of Estrogen and Estrogen-like Compounds on Atherosclerosis in Cholesterol Fed Cockerels. (27067)

W. A. PECK, W. D. WOODS, MICHAEL RAINES, A. M. DUTTON AND J. L. ORBISON

Departments of Pathology, Surgery and Radiation Biology, University of Rochester, School of Medicine and Dentistry, Rochester

Exogenous sex hormones have been observed to modify coronary atherosclerosis in experimental animals(1). Feminizing doses of estradiol benzoate will inhibit coronary atherosclerosis in cholesterol fed cockerels and will induce regression of pre-existing lesions. The mechanism of estrogen inhibition of coronary atherosclerosis is as yet unclear, though data suggest a relationship with its feminizing potency(2). Three estrogen-like compounds of low feminizing potency,* synthesized and purified by Dr. Paul Keurath, were used to study further the relationship between these effects. Two of these analogues did have an effect on the course of the experimental disease.

Methods. Forty white leghorn cockerels, one month of age, were divided into 6 experimental groups, and each bird was allocated to an individual cage. All birds received a basic diet of standard chick growing mash. The experimental diet was formulated by supplementing the growing mash with 1% cholesterol and 20% cottonseed oil. The 3 estrogen-like analogues, 2 iodo-estradiol, 2 iodo-estrone and 2 iodo-estrone acetate, all of low

* Feminizing effects of these compounds were bioassayed in ovariectomized rats by the Endocrine Laboratories, Madison, Wisc.

feminizing potency,* were injected subcutaneously in 0.5 mg doses every other day. Estradiol benzoate was given similarly in 1 mg doses.

At the end of 3 months on this regimen, the birds were sacrificed, weighed, and their combs and wattles measured and testes weighed. Autopsies were performed and tissues submitted for histologic eamination. The aortas were opened longitudinally and fixed in troughs of formalin. The hearts were fixed whole, and 5 transverse blocks were cut from each for histologic examination. All sections were stained with H & E and selected sections with oil red O to demonstrate fat.

The minimum histologic criterion for atherosclerosis was arbitrarily established as subendothelial vacuolization with elevation of the endothelial cells by accumulated fat. The total number of arteries was counted and recorded according to size (large, medium, and small) and degree of involvement assessed. Arteries of the right and left ventricle were recorded separately.

Results. The effects of the diet on growth and the feminizing effects of estradiol benzoate and the estrogen analogues were estimated by determining body weight, comb dimensions, testicular weight, and spermatogenesis and by calculating testicular weight/body weight ratios. Table 1 illustrates the effects observed in experimental birds and controls.

Table 2 is a record of the atherosclerosis observed in coronary arteries and aorta. The coronary atherosclerosis induced by the fat and cholesterol feeding involved predominately the small arteries in the subendocardial one third of the left ventricle. The vessels of the right ventricle were spared. In Table 2 it is seen that the 2 observers varied in their interpretation of how frequently atherosclerosis was present in coronary vasculature, but the variation was not so great but that clear differences could be distinguished between some of the groups. As expected, the animals given estradiol benzoate had a significantly reduced incidence of coronary atherosclerosis. The compounds 2 iodo-estradiol and 2 iodo-estrone

TABLE I. The means of measurements of primary and secondary sexual structures are recorded and indicate effect of diet and chemical compounds on sexual development.

Groups	Comb Length (cm)	Comb Height (cm)	Wattle Length (cm)	TW/BW* x 10^{-4}
Untreated	12.7	8.0	6.3	70
Cholesterol	10.1	6.3	4.7	50
Cholesterol and estradiol benzoate	5.6	3.0	1.3	5
Cholesterol and 2-iodo-estradiol	10.2	6.4	4.6	42
Cholesterol and 2-iodo-estrone	9.5	6.0	4.0	33
Cholesterol and 2-iodo-estrone acetate	9.2	5.7	3.7	25
Standard error of any mean in the column above	0.5	0.4	0.1	10

* TW/BW is abbreviation for testicular wt/body wt ratio.

TABLE II. Degree of atherosclerosis in both coronary arteries and aorta is tabulated to demonstrate effects of diet and chemical compounds.

	Coronary Arteries			Aorta
	No. of arteries counted	% of coronary arteries showing atherosclerosis		% of surface involved by atherosclerosis
		Observer 1	Observer 2	Mean of 2 observers' scores*
Untreated	—	0	0	7.0
Cholesterol	181	17.9	21.1	15.7
Cholesterol and estradiol benzoate	161	6.1	6.9	61.0
Cholesterol and 2-iodo-estradiol	168	14.4	18.6	19.0
Cholesterol and 2-iodo-estrone	190	17.1	21.1	30.4
Cholesterol and 2-iodo-estrone acetate	174	12.3	12.5	18.0
Standard error of any mean in column above	5.7	2.9	3.7	0.6

* Mean of 2 observers' scores is reported since the difference between the 2 was not significant.

had no effect on the atherosclerosis when compared with the effect of the cholesterol diet. The effect of 2 iodo-estrone acetate just failed to reach a statistical difference of P = .05 when compared with the other 2 analogues and with estradiol-benzoate. The statistical analysis was made by the method of Duncan (3). However, the effect of 2 iodo-estrone acetate lies between that of the other 2 analogues and that of estradiol-benzoate.

In contrast to the protective effect of estradiol benzoate on coronary vessels, the effect on the aorta was definitely to enhance atherosclerosis. Of the 3 analogues tested, only 2 iodo-estrone had a statistically significant enhancing effect on aortic atherosclerosis (P = .05), but the effect was less marked than that of estradiol. The other 2 analogues had no greater effect than the cholesterol diet alone.

Discussion. In this experiment estradiol benzoate produced feminization, inhibition of coronary atherosclerosis and enhancement of aortic atherosclerosis similar to reports previously made by others(4,5) and thus acted as a positive control. When the effects of the 3 estrogen analogues are compared with those of estradiol benzoate and with the cholesterol diet alone, several interesting differences are observed. The effects of the analogues on the coronary arteries did not reveal statistically significant protection by any of the 3. However, the effect of 2 iodo-estrone acetate lies between the other 2 analogues on the one hand and estradiol benzoate on the other. Due to the lack of statistical significance in either direction, this result is not clear-cut, but suggests a difference between 2 iodo-estrone acetate and both estradiol benzoate and the other analogues.

In contrast to the observations on the coronary arteries, estradiol benzoate had a distinctly enhancing effect on aortic atherosclerosis. Similarly 2 iodo-estrone had a statistically significant effect in enhancing aortic atherosclerosis. This effect of 2 iodo-estrone was not so great as estradiol benzoate, but was definitely greater than the cholesterol diet alone. The other 2 analogues had no effect on the aortic lesions.

Comparison of the feminizing effects of the 3 analogues reveals 2 iodo-estradiol to have no identifiable effects over those general growth effects of the experimental diet alone. The other 2 analogues appear to have slight feminizing capacity.

When the above effects are correlated, it is seen that 2 iodo-estrone acetate resembles estradiol benzoate more closely than the other 2 analogues in its effects on coronary atherosclerosis whereas 2 iodo-estrone most closely resembles estradiol benzoate in its effect on aortic atherosclerosis. Since these two compounds have only limited feminizing effect and yet differ definitely from one another in their effects on coronary arteries and aorta, it is suggested that the effects of these compounds on coronary atherosclerosis and on aortic atherosclerosis are mediated by differences in molecular configuration other than those inducing feminization. Further, it is suggested that the differences in molecular configuration between 2 iodo-estrone and 2 iodo-estrone acetate are manifested by differences in their comparative activities on the aorta and coronary arteries.

Summary. Coronary and aortic atherosclerosis were studied in white leghorn cockerels fed an atherogenic diet and treated with parenteral estradiol benzoate or synthetic estrogen compounds of lower feminizing potency. The estradiol benzoate gave the expected feminization and protection against coronary atherosclerosis and enhanced thoracic aortic atherosclerosis. The 2 iodo-estrone acetate gave some suggested protection to the coronary vasculature, but was not clearly different from the other analogues in this respect. The 2 iodo-estrone enhanced aortic atherosclerosis and was significantly different in this effect from the other analogues. These observations suggest that the effects on atherosclerosis of the coronary arteries and aorta are mediated through molecular configurations different from those mediating feminization.

1. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., *Circulation*, 1952, v6, 276.

2. Stamler, J., Pick, R., Katz, L. N., *Ann. N. Y. Acad. Sci.*, 1956-57, v64, 596.

3. Duncan, D. B., *Biometrics*, 1955, v2, 1.

4. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., *Circulation*, 1956, v6, 858.

5. Rivin, A., Dimitroff, S., *ibid.*, 1954, v9, 533.

Received September 22, 1961 P.S.E.B.M., 1961, v108