Dissociation of Feminizing Activity and Protective Effect of Steroids in Rats Fed a Thrombogenic Diet (33779)

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The greater incidence of atherosclerotic heart disease in men than in premenopausal women, and the reduction of serum lipids by estrogen administration to man and experimental animals, have prompted a search for chemical modifications of the estrogen molecule that reduce the undesirable, feminizing side effects and yet retain the hypolipemic activity (1). A number of steroids have been synthesized which will effectively lower the serum cholesterol of normal or hypercholesterolemic rats and which will elicit little response in conventional tests for estrogenicity (1). It is not known whether these compounds of reduced estrogenicity will be of therapeutic value for human atherosclerosis.

The studies of Renaud (2) on the effect of feeding a thrombogenic diet to rats revealed a sex influence analogous to that in humans. Female rats had a lower serum cholesterol, less incidence of thrombosis and a lower mortality rate than castrated females, normal or castrated males.

The implication that estrogen administration to male rats would alleviate the deleterious effects of the thrombogenic diet was the basis for the present study testing the comparative effectiveness of a natural estrogen with a synthetic steroid possessing equivalent hypocholesterolemic activity, but with a low degree of estrogenicity.

Methods and Materials. Male hooded rats (Quebec Breeding Farms) were used. The thrombogenic diet consisted of (%): ground Purina lab chow, 50; cholesterol, 5; cholic acid, 2; and butter, 43. Food and water were given *ad libitum*.

The steroid preparations selected for comparison were *dl*-13β-ethyl-3-methoxy-8α-gona-1,3,5(10)-trien-17β-ol (Wy-3359) (3) and equine conjugated estrogen (Premarin, Ayerst). The synthetic compound, of low estrogenicity, when administered as the racemate, has an oral activity of about half that of *d*-estrone or *d*-β-estradiol in reducing serum cholesterol, phospholipids, and triglycerides (4). The dietary concentrations were 25 mg/kg for Wy-3359 and 15 mg/kg for Premarin; the daily intake of steroids averaged 0.25 and 0.15 mg, respectively. Two experiments, each of approximately 7-months duration, were conducted.

In the first experiment, 45 male weanling rats were fed the thrombogenic diet for 1 month, then divided into three groups of 15 with equal average body weights and serum cholesterol levels. One group was continued on this basal diet; the other two received diets containing either the natural preparation or the synthetic steroid. The experiment was terminated after 28 weeks when only a few animals remained in each group. All animals were examined at autopsy for gross lesions. Selected organs were weighed and sections were examined histologically.

In the second experiment, 3 groups of 29, 30, and 30 rats with an average weight of 100 g were each fed one of the three experimental diets for 28 weeks. After 2 months, 6 typical rats from each group were selected for analysis of liver lipid and cholesterol. Similar analyses were run on the animals that survived the experiment. The total lipids of the livers were extracted by the procedure of Bixby *et al.* (5).

In both experiments serum cholesterol was determined biweekly. Cholesterol of the serum and of liver extracts were determined by the procedure of Zlatkis *et al.* (6).

Results. Serum cholesterol. In rats fed the unsupplemented thrombogenic diet, serum cholesterol values increased from initial values of 80 mg/100 ml to 300–500 mg/100 ml by the end of the first month. During the first 3 months of both experiments the average serum cholesterol of the groups receiving the steroids fluctuated between 200–350 mg/100 ml; the control groups, 500–600 mg/100 ml.

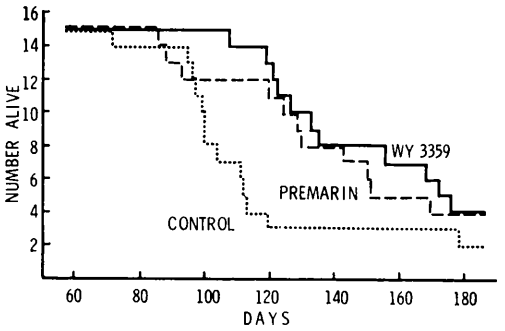

FIG. 1. Effect of Premarin and Wy-3359 on the survival time of rats fed a thrombogenic diet (Expt. 1).

The dietary concentrations of the natural and the synthetic steroid produced equal hypocholesterolemic effects. From the third month on, averages of all the groups fluctuated widely. This was caused by sudden increases to extremely high levels, up to 2000–5000 mg/100 ml in individual rats. Most of these rats died within the next week. Thus, the average degree of hypercholesterolemia depended on the number of such animals alive at the time of blood sampling. The animals of the control group that survived the 7-month experiment had low serum cholesterols, not different from those of the steroid-treated rats (Table IV). These survivors had had relatively low serum cholesterols throughout the experiment.

Survival time. Steroid administration significantly increased the survival time of the rats fed the thrombogenic diet. In the first experiment, the average survival time of the control group was significantly less at 118 ± 10 days compared to 152 ± 7 days and 145 ± 8 days for groups receiving Wy-3359 and Premarin, respectively (Fig. 1). The synthetic preparation and the natural steroid preparation, administered at equal hypocholesterolemic doses, were of equal benefit in prolonging life.

The results of the second experiment closely agreed with those of the first. From the fourth month on, there was a statistically significant greater number of rats alive in the steroid-treated groups than in the control (Table I).

Autopsy findings. At autopsy, all animals showed extremely enlarged, pale yellow livers. Necrotic areas were seen in the hearts and kidneys, and the lungs of most of the animals showed varying degrees of infection. The hooded rat is less prone to the severe pulmonary infections commonly found in white rats maintained under comparable dietary conditions. In those rats that died during the experiment, there was gross enlargement of the spleen, and testicular atrophy was found in the rats receiving Premarin and Wy-3359 (Table II). In contrast, in animals that survived the experiment, spleen weights were normal, and a reduction in testes weights was found only in the rats receiving Premarin (Table III).

Microscopic examination of the lungs, spleen, and liver presented a picture of a

TABLE I. Effect of Steroid Treatment on Survival Time (Expt. 2).[a]

Group (days)	Control	Wy-3359	Premarin
Av survival time (days ± SE):	117.3 ± 10.7	150.9 ± 10.5[b]	160.6 ± 10.5[b]
		Fraction alive	
75	14/23	19/24	22/24[c]
125	11/23	17/24[c]	17/24[c]
155	7/23	17/24[c]	16/24[c]
180	6/23	12/24[c]	14/24[c]
206	5/23	10/24[b]	13/24[b]

[a] *t* test for survival time; chi square test for fraction alive.
[b] Statistically significant, $p = 0.05$.
[c] Statistically significant, $p = 0.01$.

TABLE II. Relative Organ Weights of Survivors and Fatalities (Expt. 1).

Steroid	No. of rats	Body wt. (g)	(g/100 g of body wt.) Testes	Spleen
None	13 F[a]	148	1.51	2.59
	2 S[b]	245	1.34	1.06
Wy-3359	12 F	135	0.38	2.66
	3 S	190	1.17	0.67
Premarin	12 F	124	0.26	2.30
	3 S	200	0.49	0.73
None (chow fed)	3	250	1.25	0.41

[a] Fatalities.
[b] Survivors.

severe generalized lipidosis. The heart lesions consisted of varying degrees of necrosis with multiple thrombi in the small vessels. The primary change in the kidneys was a tubular degeneration with extensive pigment deposition. The testicular degeneration characteristic of estrogen administration was present in the fatalities of the Wy-3359 group and in all rats fed Premarin.

Liver lipid and cholesterol. There was no difference in the total lipid and cholesterol contents of the livers of the three groups after 2 months on the thrombogenic diet (Table IV). After 7 months total liver lipids were slightly but significantly lower in the groups receiving steroids. Liver cholesterol was significantly lower only in the Premarin group. The massive amounts of lipid present, would tend to obscure small differences.

Discussion. The natural conjugated estrogen preparation and the synthetic preparation, when fed at equivalent hypocholesterolemic doses, were equally effective in prolonging the lives of hooded rats fed the thrombogenic diet. The greater resistance of the female rats compared to that of males and castrated females in the study of Renaud (2) appears to be related to the hypolipemic activity of endogenous steroid. In a recent oral presentation,[1] Renaud has further reported that the inclusion of Premarin in a hyperlipemic diet reduced the severity of the thrombotic lesions initiated by the intravenous injection of bacterial lipopolysaccharide.

The results of the present study indicate that the protective activity of an estrogen against a thrombogenic diet, like hypocholesterolemic activity, may be dissociated to a great degree from the feminizing activity. Pronounced atrophy of the testes was found in all animals administered Premarin while the testicular weights of the Wy-3359-fed rats that survived the experiments were not significantly different from those of the controls. The feminizing activity of the weakly estrogenic synthetic steroid was evident in the reduced testes weights of the rats that died during the experiment. Presumably, in the terminal stages of the dietary-induced disease, the extremely fat-infiltrated liver progressively loses its ability to inactivate the orally administered steroid permitting more and more to reach the target organs.

TABLE III. Effect of Steroids on Relative Organ Weights of Survivors (Expt. 2).

Time (months)	Steroid	No. of rats	Body wt. (g)	(g/100 g of body wt. ± SE) Liver	Testes	Spleen
2	None	6	221	11.2 ± 0.7	1.35 ± 0.05	0.47 ± 0.06
	Wy-3359	6	223	9.9 ± 0.4	1.17 ± 0.12	0.40 ± 0.05
	Premarin	6	201	9.3 ± 0.4	0.73 ± 0.11[a]	0.43 ± 0.04
7	None	5	312	11.8 ± 0.7	1.05 ± 0.05	0.49 ± 0.04
	Wy-3359	10	253	12.8 ± 0.4	0.97 ± 0.13	0.51 ± 0.02
	Premarin	13	230	12.6 ± 0.5	0.47 ± 0.07[a]	0.58 ± 0.03

[a] Statistically lower than control, $p = 0.01$, t test.

[1] Twentieth Ann. Meeting Can. Cardiovascular Soc.; Abstr. *in* Can. Med. Assoc. J. 98, 119 (1968).

TABLE IV. Effect of Steroids on Liver Lipid and Cholesterol (Expt. 2).

Time (months)	Steroid	No. of rats	Liver		Serum cholesterol (mg/100 ml ± SE)
			Lipid (% ± SE)	Cholesterol (% ± SE)	
2	None	6	29.9 ± 1.7	13.2 ± 0.3	506 ± 38
	Wy-3359	6	33.6 ± 2.0	15.1 ± 0.6	292 ± 26[b]
	Premarin	6	29.7 ± 1.5	13.3 ± 0.5	294 ± 14[b]
7	None	5	28.1 ± 1.2	14.1 ± 0.6	282 ± 44
	Wy-3359	10	25.7 ± 0.5[a]	13.9 ± 0.4	244 ± 23
	Premarin	15	25.5 ± 0.8[a]	12.8 ± 0.3[a]	340 ± 50

[a] Statistically lower than control, $p = 0.05$, t test.
[b] Statistically lower than control, $p = 0.01$, t test.

Death presumably occurs in these animals when the mechanism for control of fat transport and utilization can no longer cope with the constant dietary stress. This failure is evidenced by the precipitous rise in serum cholesterol shortly before death and by the gross hypertrophy of the spleen as it responds in its phagocytic functions of removing and storing excess circulating lipid.

The small differences in liver lipid content between control and steroid-fed rats suggests that the prolongation of the life of rats fed a thrombogenic diet by the administration of hypocholesterolemic steroids does not involve a protection against fat infiltration of the liver.

Summary. A natural conjugated estrogen preparation, Premarin, and a synthetic steroid, *dl*-13β-ethyl-3-methoxy-8α-gona-1,3,-5(10)-trien-17β-ol, at dietary concentrations of equal hypocholesterolemic activity, were equally effective in prolonging the lives of male rats fed a thrombogenic diet. The insignificant effect of the synthetic steroid on testicular weights demonstrated that antithrombogenic activity was dissociated to a large degree from feminizing activity.

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