An Orally Active Hypocholesteremic Steroid with Reduced Uterotrophic Effect. (30714)

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Numerous investigators(1-3) have noted abnormal blood lipid patterns in patients with atherosclerotic heart disease which appear to be shifted towards or to normal by estrogens. As a consequence efforts have been made to dissociate the lipid-shifting from the feminizing effects of these substances(4-6). Following is a discussion of an estrone derivative that exhibits these properties experimentally.

Methods. The hypocholesteremic activity was estimated following oral administration of the compounds to intact mature male rats, whose weights generally averaged 225 to 275 g, daily for 4 days. On the fifth day blood was taken by cardiac puncture, the serum separated, and the cholesterol subsequently determined by the macro-procedure of Turner and Eales(7) with *p*-toluenesulfonic acid as the catalyst in the Lieberman-Burchard procedure.

As a measure of estrogenicity, the uterotrophic effects were estimated in weanling female rats given the test compounds daily for 3 days by intubation. On the fourth day the uteri were excised, blotted dry and weighed on a micro-torsion balance.

Since the hypocholesteremic and uterotrophic activities are unlike properties, the hypocholesteremic effects have been arbitrarily expressed as the amount of compound, in mg, required to reduce serum cholesterol by 33% (ED_{33}). The minimal amount of steroid required to increase the uterine weights by 50 mg over that of the controls has been expressed as an mD-50 value. The magnitude of these responses is highly significant in each instance ($P = <0.001$). In addition, the ratio of the hypocholesteremic ED_{33} to the uterotrophic mD-50 is a measure of the relative separation of the two activities, the lower the ratio the more desirable the hypocholesteremic:uterotrophic dissociation.

Results. Results from multiple-level bioassays on reference estrogens, estrone (I), estrone methyl ether (III), and on several synthetic steroids of interest are summarized in Table I.

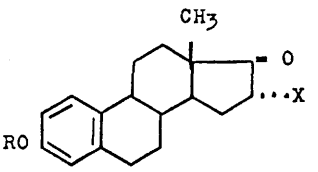
On the basis of these evaluations it may be seen that estrone (I) (ratio = 50), estrone methyl ether (III) (ratio = >3.2), and Premarin® (II) (ratio = 20) are more uterotrophic than they are hypocholesteremic. 16α-Chloroestrone methyl ether (IV), which appears not to have been successful in preliminary clinical trials(8), was also somewhat more uterotrophic than hypocholesteremic under these conditions of evaluation (ratio = 2.4).

17-(3-Hydroxy-1-propynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 17α-hydrocinnamate (VII) was hypocholesteremic at 12 mg per kg per day, but exhibited a flat uterotrophic dose response curve, illustrated graphically in Fig. 1, and yielded the lowest hypocholesteremic:uterotrophic ratio, 0.015, of any estrogen that we have studied. This favorable ratio resulted more from the poor uterotrophic response which accompanied increasing doses rather than from an absolute separation of the hypocholesteremic and uterotrophic activities. Two representative closely related estrone derivatives, the hydroxybutynyl compound V and the hydroxypropyl compound VI of the Table, showed estrogenicity in the usual range and the resulting hypocholesteremic:uterotrophic ratios were near unity (ratios of 1.6 and 0.2, respectively).

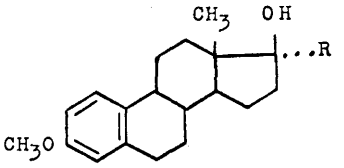
Thus, compound VII manifests the best hypocholesteremic:uterotrophic separation of any estrogen that we have evaluated. Whether this experimental separation will carry over to clinical usefulness for favorable alteration of blood lipid parameters and protection against recurrent coronary attacks with minimal manifestations of estrogenicity remains to be determined.

Summary. A series of reference and ex-

TABLE I. Hypocholesteremic and Uterotrophic Effects of Some Experimental and Reference Steroids.



I: R = X = H
III: R = CH_3, X = H
IV: R = CH_3, X = Cl



V: R = $HOCH_2CH_2C \equiv C$
VI: R = $HOCH_2CH_2CH_2$
VII: R = $C_6H_5CH_2CH_2COOCH_2C \equiv C$

Steroid	Hypocholesteremic ED_{33} (mg/kg/day × 4)	Uterotrophic mD-50 (mg/kg/day × 3)	Ratio
I: Estrone	2.5	.05	50
II: Premarin®*	4.2	.18	20
III: Estrone methyl ether	1.6	<.5	>3.2
IV: 16α-chloroestrone methyl ether(8)	2.6	1.1	2.4
V: 17-(4-hydroxy-1-butynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol(9)	32	20	1.6
VI: 17-(3-hydroxypropyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol(10)	2.4	12	.2
VII: 17-(3-hydroxy-1-propynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 17α-hydrocinnamate	12	800	.015

* Premarin® is an extract of pregnant mare's urine consisting of conjugated equine estrogens.

perimental estrogens has been evaluated in terms of the amount required to reduce total serum cholesterol 33% in mature male rats and the amount required to increase the uterine weights of weanling female rats 50 mg over those of the controls. The resultant hypocholesteremic : uterotrophic dissociations were: estrone 50, Premarin® 20, estrone methyl ether >3.2, 16α-chloroestrone methyl ether 2.4, 17-(4-hydroxy-1-butynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 1.6, 17-(3-hydroxypropyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 0.2, and 17-(3-hydroxy-1-propynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 17α-hydrocinnamate 0.015. The hydroxy propynyl compound thus has the best dissociation of any steroid noted.

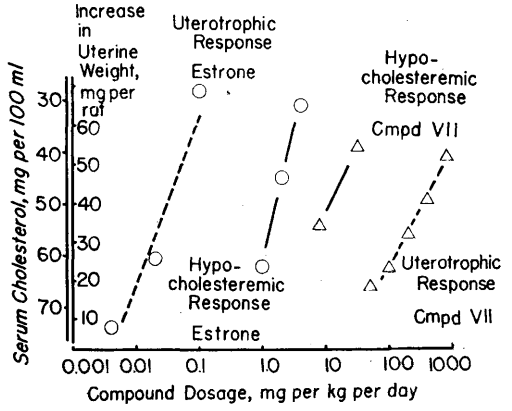


FIG. 1. Hypocholesteremic and uterotrophic responses to increasing doses of esterone and of 17-(3-hydroxy-1-propynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol 17α-hydrocinnamate (cmpd VII).

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Peripheral Arterial and Renal Venous Angiotensin Concentration.* (30715)

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Investigations of the renal pressor proteinaceous material called renin have clarified its enzymatic characteristics(1,2). The active product, angiotensin, has been purified, synthesized, and biologically characterized, but its role in health and disease is still controversial(3). Its pressor characteristic has suggested it to be a link in the etiology of hypertension, in particular, renovascular hypertension(4); yet its aldosterone stimulating property has suggested a role in the control of sodium balance(5). If angiotensin is of importance either in hypertension or in salt metabolism, angiotensin blood concentrations would be useful in defining its role. Prior measurements have been made without regard to salt intake largely in patients or animals with various hypertensive disorders(6,7,8,9, 10). Under these conditions, blood concentrations have been reported to be both elevated and normal. The study reported herein was designed to overcome theoretical disadvantages present in other studies by 1) rigorously controlling sodium intake and 2) by sampling renal venous blood, presumably the source of circulating angiotensin. In addition, renal venous concentrations were compared to peripheral arterial concentrations in various patients.

Methods. A. Patient material. Each patient was studied on a metabolic balance ward with the exceptions mentioned. A constant diet containing less than 10 mEq sodium was given throughout the study period. All medications were discontinued and none were administered during study. Patients were either normal (arterial pressures normal; response to sodium restriction normal) or hypertensive. Patients with hypertension had either renovascular lesions demonstrated by aortography or essential hypertension. After each patient came into balance as demonstrated by stable weights and urinary sodium for three days, a percutaneous renal vein catheterization study was performed using Seldinger technique(11) combined with cinefluorography. Catheter location was checked by the rapid injection of 2 ml 50% hypaque which allowed visualization and recording of the renal vein outline (Fig. 1). Fifty to 100 ml of blood were aspirated from each renal vein for angiotensin assay. Afterwards each patient was placed on 12 g of sodium chloride daily (>200 mEq Na) while keeping the diet otherwise constant. On the high sodium intake renal vein catheterization was repeated after body weight and urine sodium were stable. Therefore, in each patient, renal venous samples were analyzed for angiotensin concentration during sodium deprivation and during sodium surfeit.

Peripheral venous blood was obtained twice weekly for blood urea nitrogen, hemoglobin, hematocrit and serum bicarbonate, sodium, potassium, and chloride. An aliquot of 24-hour urine samples was analyzed daily for sodium, potassium and creatinine. Arterial blood pressures were measured every six hours in the recumbent position. In cases identified by Table II a percutaneous renal

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