cation confirms but does not explain this observation and proliferation of the intima of the aorta containing numerous foam cells (Fig. 1, B) demonstrates the damaging effect of simultaneous feeding of cholesterol and dimethylpolysiloxane. The mechanism by which ingested phenylmethylpolysiloxane prevents severe hypercholesterolemia and the proliferative lesions of the aorta is also obscure, but one may postulate that the phenyl group of this more soluble silicone fluid enhances the transport of triglyceride and cholesterol by the reticuloendothelial system (10).

Summary. Addition of dimethylpolysiloxane to a diet containing 2% cholesterol does not alter hypercholesterolemia and cholesterol content of the liver of rabbits. However, it increases cholesterol content of the aorta and produces atheromatous lesions of the intima. Addition of phenylmethylpolysiloxane to a diet containing 2% cholesterol prevents severe hypercholesterolemia and increases cholesterol content of the liver. It lowers the cholesterol content of the aorta and prevents atheromatous proliferation.

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Received April 21, 1961. P.S.E.B.M., 1961, v107.

Estrogen-Progesterone Antagonism on Endometrial Carbonic Anhydrase Activity.* (26652)

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There is ample evidence concerning estrogen-progesterone antagonism in the uterine endometrium. Much has been demonstrated by the histological test based on the proliferative changes in the uterus, the response being rated on the semiquantitative scale. However, little has been done to utilize enzyme determinations in the endometrium as an indicator of hormonal antagonism.

Lutwak-Mann and Adams(1) have studied the effect of stilbestrol on development of progestational activity by the measurements of uterine carbonic anhydrase and progestational proliferation. They observed that in the enzymic test stilbestrol prevented progestational development, and noted that with few exceptions there was good agreement between the enzymic and histological test in evaluating the antagonism.

More recently a work of Miyake and Pincus(2), concerning the antiprogestational activities of estrogens, namely estradiol, estrone, estriol and stilbestrol, in Clauberg rabbits provided quantitative evidence of the antagonism by estimating carbonic anhydrase activity in the uterine endometrium. This was also associated extremely well with proliferative changes (ratio of glandular to total mucosal area). They suggested thereby the superiority of estimation of uterine carbonic anhydrase as a quantitative test for antiprogestational activity.

The present investigation was made to as-

* This investigation was supported by research grant from the Population Council, Inc., Rockefeller Institute. Some hormones were generously supplied by Teikoku Zoki Co. (Japan) through the courtesy of Dr. S. Matsushima.

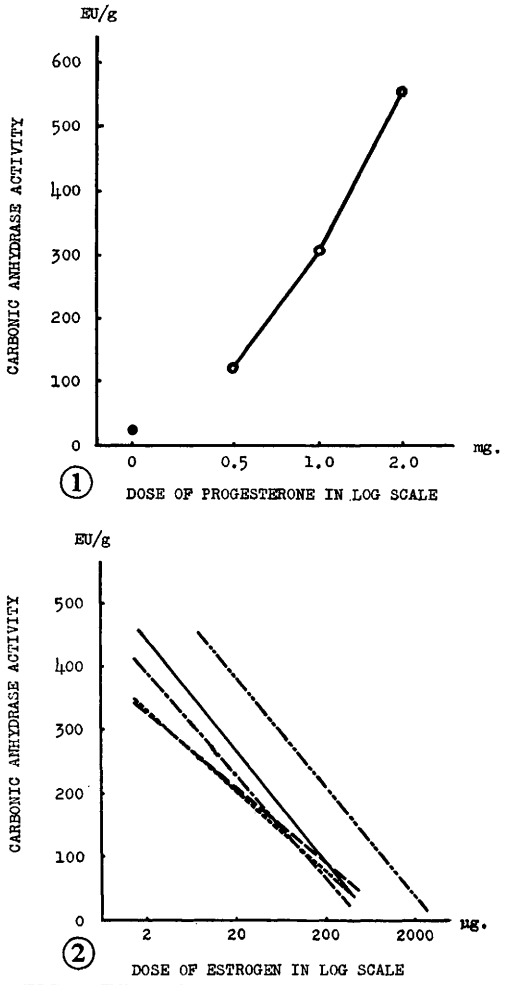

FIG. 1. Effect of progesterone on activity of endometrial carbonic anhydrase in Clauberg rabbits. Progesterone was given in amounts ranging from 0.5 mg to 2.0 mg per animal in 5 divided doses at daily intervals by subcutaneous injection. Carbonic anhydrase activity was expressed as EU/g wet tissue. Five rabbits in each group. ●, control; ○, treated with progesterone.

FIG. 2. Dose-response regression lines of estrogens for endometrial enzymic response to 2.0 mg progesterone per animal. The slope is negative.

——— : Estradiol $Y = 491 - 174 \cdot \log X$
— — —: Ethinyl estradiol $Y = 371 - 122 \cdot \log X$
--------: Hexestrol $Y = 373 - 129 \cdot \log X$
——— : Estrone-3-methyl ether $Y = 441 - 162 \cdot \log X$
——— : Estriol-3-methyl ether $Y = 603 - 172 \cdot \log X$

certain whether the following estrogens are capable of affecting the enzymic response to progesterone in the uterine endometrium; estradiol, ethinyl estradiol, hexestrol, estrone-3-methyl ether and estriol-3-methyl ether.

Material and methods. Immature albino rabbits, approximately 1.5 kg in weight, were used. All of the animals were primed with once daily subcutaneous injections of 5 μg estradiol each for 6 days, according to the Clauberg method(3). The animals were then injected subcutaneously with progesterone and/or test compounds, once daily for 5 days. The test compounds dissolved in sesame oil to graded concentration were used and injection volume was 0.2 cc per animal daily.

The rabbits were sacrificed on the second day after last injection. The uteri were isolated after bleeding thoroughly from the carotid artery. The endometrium was dissected and homogenized with cold distilled water. The homogenate was centrifuged at 2,000 r.p.s. for 10 minutes and the supernatant was used for determination of enzyme activity. The enzyme activity of the extract was assayed colorimetrically by modifying the Philpot and Philpot method(4), one enzyme unit (E.U.) being defined as described in the method of Wilbur and Anderson(5).

$$\frac{To - T}{T} = \text{one unit of activity}$$

where "To" is reaction time without enzyme and "T" is the same with enzyme.

As previously demonstrated by Miyake and Pincus(2), this definition of enzyme unit is also suitable in the colorimetric method of Philpot and Philpot. A linear relationship between (To–T)/T and concentration of enzyme is obtainable in keeping "To" in 60 to 90 seconds and adjusting to get "T" in the range of 10 to 40 seconds.

Results. Activity of endometrial carbonic anhydrase in response to progesterone. In these experiments, estrogen primed rabbits were given amounts of from 0.5 mg to 2.0 mg of progesterone by subcutaneous injection. The results are recorded in Fig. 1. These findings agree with the observations of Lutwak-Mann(6) and Pincus *et al.*(7).

Effects of estrogens on augmented carbonic anhydrase activity caused by progesterone. In experiments to ascertain the antagonistic effect of estrogens, 5 compounds (estradiol, ethinyl estradiol, hexestrol, estrone-3-methyl ether and estriol-3-methyl ether) were employed. Progesterone and estrogen were given simultaneously in 5 divided doses at

TABLE I. Inhibitory Effects of Estrogens on Augmented Activity of Endometrial Carbonic Anhydrase Caused by Progesterone and Percent Inhibition of Progesterone by Estrogen.

Group	Series of hormone	Dose (mg/animal) of Progesterone	Dose (mg/animal) of Estrogen	No. of rabbits	EU/g wet tissue	% inhibition of progesterone by estrogen
1	Control	0	0	5	23 ± 2*	
2	Progesterone	2.0	0	5	554 ± 18	
3	Estradiol + progesterone	2.0	.2	5	89 ± 6	88
			.02	5	270 ± 23	54
			.002	4	436 ± 35	22
4	Ethinyl estradiol + progesterone	2.0	.2	5	110 ± 10	84
			.02	5	174 ± 23	72
			.002	4	358 ± 66	37
5	Hexestrol + progesterone	2.0	.2	5	77 ± 2	90
			.02	5	202 ± 16	66
			.002	5	335 ± 38	41
6	Estrone-3-methyl ether + progesterone	2.0	.2	4	77 ± 13	90
			.02	5	217 ± 24	64
			.002	5	400 ± 47	29
7	Estriol-3-methyl ether + progesterone	2.0	2.0	5	37 ± 3	97
			.2	5	203 ± 34	66
			.02	5	381 ± 28	33
			.002	4	426 ± 36	24

* Mean ± stand. error.

Percent inhibition is expressed as $\frac{Rp - Re}{Rp - C} \times 100$; Rp = uterine enzymic response to progesterone alone; Re = uterine enzymic response to combination of progesterone and estrogen; C = control level.

daily intervals by subcutaneous injection into the neck of the animal. The standard dose of progesterone was 2.0 mg and estrogen doses varied as indicated in Table I.

When estrogens were given in a dose of 0.2 mg along with 2 mg of progesterone, the uterine endometrium had a much decreased carbonic anhydrase activity except in the case of estriol-3-methyl ether (Table I). Percent inhibition of progesterone by each estrogen was 88% by estradiol, 84% by ethinyl estradiol, 90% by hexestrol and estrone-3-methyl ether. These estrogens exhibited almost equal potency in degree of inhibition. However, in the case of estriol-3-methyl ether, enzyme activity was relatively high, an inhibition of 66% being observed. A total of 2.0 mg was necessary for a 97% inhibition of the effect of 2 mg of progesterone.

The dosage response curves (Fig. 2), show the lesser effectiveness of estriol-3-methyl ether; the curves for the 4 other estrogens are quite similar, one to another.

Effect of estrogen on activity of endometrial carbonic anhydrase. Table II presents uterine enzymic responses to these estrogens. Estrogens only were given in a dose of 0.2 mg or 2.0 mg per animal. No significant increase in endometrial carbonic anhydrase above the control was found in any instance. Therefore, it is obvious that estrogen itself is not capable of affecting the activity of endometrial carbonic anhydrase.

Discussion. These results show that estrogens we have used can prevent the augmented activity of uterine carbonic anhy-

TABLE II. Effects of Estrogens on Carbonic Anhydrase Activity of Uterine Endometrium.

Series of estrogen	Dose (mg/animal)	No. of rabbits	EU/g (wet tissue)
Control	.2	5	23 ± 2
Estradiol	.2	5	32 ± 8
Ethinyl estradiol	.2	3	27 ± 4
Hexestrol	.2	5	34 ± 9
Estrone-3-methyl ether	.2	4	32 ± 7
Estriol-3-methyl ether	2.0	4	33 ± 8

Results expressed as mean ± stand. error. Each estrogen was injected subcut. in 5 divided doses at daily intervals.

drase caused by progesterone. It seems that the antiprogestational ability of estrogen is almost the same in estradiol, ethinyl estradiol, hexestrol and estrone-3-methyl ether, but approximately 1/10 of the others in estriol-3-methyl ether, if given systemically. From the data, a linear log dose response relation of 2 antagonistic hormones was obtained in amounts ranging from 0.002 mg to 0.2 mg of estradiol, ethinyl estradiol, hexestrol and estrone-3-methyl ether and in doses of 0.02-2.0 mg in estriol-3-methyl ether, the regression about the line being highly significant. Thereby, quantitative differences in the potency of these estrogens to prevent progestational development may be provided.

Previously, Miyake and Pincus(2) showed that other estrogens (estradiol, estriol, estrone and stilbestrol) suppressed the enzymic response to progesterone, the extent of which correlated extremely well with the degree of pseudo-pregnant proliferation. The antiprogestational activities of these estrogens were all approximately the same. In their experiments, almost the same procedure as that reported here was employed. The response regression line of estradiol on progestational development is in satisfactory agreement with our data.

Considering that estrone and estriol were shown, according to them, to be the same in antagonistic potency, it would be expected that inhibitory abilities of estrone- and estriol-3-methyl ether would be the same because of certain similarities in chemical properties. However, this is not the case in our experiments. The results indicate a marked difference in the abilities of estrone- and estriol-3-methyl ether to affect the action of progesterone. Estriol-3-methyl ether seems to have approximately 1/10 the potency of estrone-3-methyl ether if the comparison is made from our regression line.

The authors are indebted to Professor T. Suzuki for many helpful suggestions and to Dr. K. Hirai for assistance throughout this study. We also wish to thank Dr. G. Pincus, Worcester Foundation for Experimental Biology, and Dr. W. O. Nelson, Population Council, Inc., Rockefeller Institute, for kindly supporting this work.

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Received April 24, 1961. P.S.E.B.M., 1961, v107.

Inhibitory Effect of Ammonium Ions on Influenza Virus in Tissue Culture. (26653)

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While examining a series of pyrimidines for antiviral activity in a tissue culture system, it was observed that one compound exhibited a marked inhibition of the cytopathic effect (CPE) of influenza virus. Subsequent studies with other preparations of the same compound showed varying degrees of activity, and in some cases a complete lack of activity. This inhibitory effect was eventually shown to be due entirely to trace amounts of ammonium ion present in the active preparations as an impurity. Further studies have shown that comparable results can be obtained with a number of inorganic and organic ammonium salts. This report describes in detail the virus inhibition and discusses possible explanations for the observed effect. It is also shown that under normal growth conditions sufficient ammonia may be accumulated in the medium to pro-