nies from mixtures where bacteriophage P10 susceptibility could segregate were always sensitive and 2) upon continued subculture, the parental types were recovered from the prototrophs. The first point conclusively ruled out reciprocal feeding by a mixed population of cells because the resistant component could not be demonstrated as a discrete unit, indicating that the interaction was intracellular. Both lines of evidence argued against haplophase recombination because 1) bacteriophage P10 susceptibility did not segregate and 2) the prototrophs subsequently yielded parental dissociates. The remaining possibilities were heterokaryosis and parasexuality(6). The latter hypothesis would predict that nonparental types as well as parental types should be recovered from the prototrophs. Inasmuch as nonparental types have not been found, the only hypothesis which was consistent with all of the data was heterokaryosis.

Our data indicated that 2 factors determined heterokaryotic compatibility in *S. coelicolor,* as was found to be the case for heterokaryotic compatibility in *Neurospora crassa* (7). At least one of the compatibility factors arose by mutation because S207 and S125 displayed different compatibility responses even though these two strains had a common ancestor. It is not possible at this time definitely to conclude that the factors controlling heterokaryotic compatibility in *S. coelicolor* represent nuclear genes.

Summary. Heretofore, complementary pairs of growth-factor dependent mutants of *Streptomyces coelicolor* have interacted to yield colonies which grow readily in the absence of added supplements. Recently however certain combinations have been found which did not interact successfully. This failure of nutritionally dependent mutants to form growth-factor independent growth was not the result of allelism, but of a compatibility system controlling heterokaryon formation. The compatibility system was apparently determined by two factors, one of which probably arose by mutation.

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Anti-Progestational Activity of Estrogens in Rabbit Endometrium*† (24390)

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Established ability of estrogens to inhibit progestin-induced endometrial proliferation has been thoroughly reviewed by Courrier(1). This anti-progestational effect has been determined by histological methods, and its demonstration has been either qualitative or roughly quantitative. With development of the Lutwak-Mann test(2) for progestin-induced endometrial carbonic anhydrase increase, as a quantitative assay in the rabbit (3), we felt that accurate quantitation of the anti-progestational effect might also be achieved. We had previously shown that quantitative estimates of progestational potency made by carbonic anhydrase measurement correlated extremely well with concomitant measurements of the degree of pseudopregnant proliferation(3,4). Accordingly, in studying the estrogen effect we have measured in samples of the same uteri both carbonic anhydrase concentration and degree of pseu-

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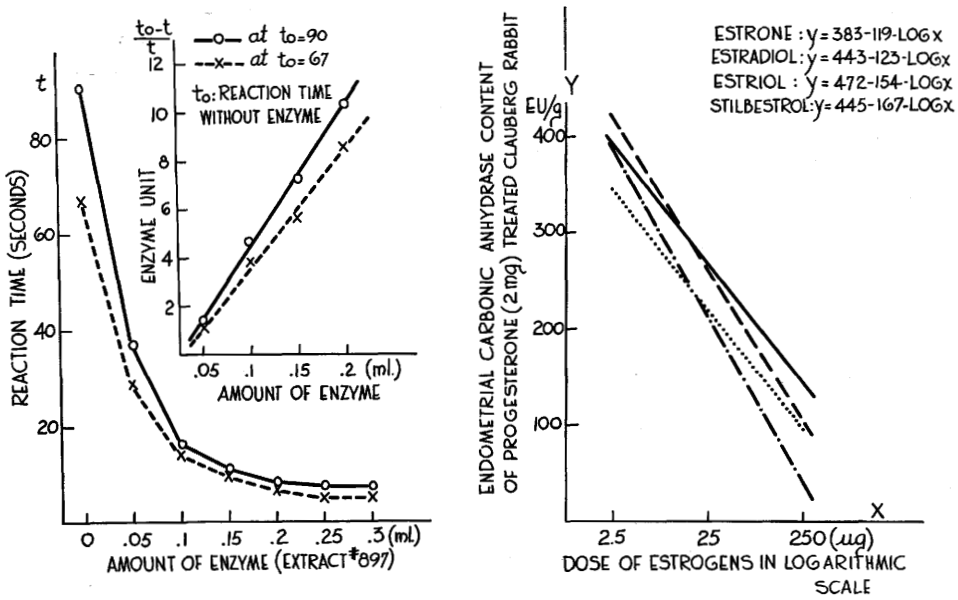

FIG. 1 (left). The relationship between enzyme unit and amount of enzyme.
FIG. 2 (right). Dose-response regression lines of estrogens on anti-progestational activity.

dopregnant proliferation. We present here the effects on endometrial carbonic anhydrase and pseudopregnant proliferation of: (a) 4 estrogens—estrone, estradiol-17β, estriol and stilbestrol, (b) various dosages of progesterone alone and in combination with fixed dosage of estradiol-17β and (c) various dosages of 4 estrogens in combination with a standard dose of progesterone.

Methods. Following the Clauberg method (5), immature rabbits weighing approximately 1.5 kg were injected subcutaneously once daily with 5 μg of estradiol for 6 days. After this period of estrogen-priming, rabbits were injected subcutaneously once daily with various doses of progesterone and/or estrogen. Progesterone in 0.2 ml volume was injected at the neck, and estrogen in 0.1 ml at the center of back. The animals were sacrificed 24 hours after last injection. All hormones were dissolved in Sesame Oil U.S.P. The procedures for measurement of endometrial carbonic anhydrase content are the same as those described previously(3,4). In the estimation of enzyme activity, however, the following definition of an enzyme unit was used.

$$\text{EU/g wet tissue} = \frac{t_o - t}{t} \times \frac{1000}{C \cdot V}$$

EU = Enzyme unit
t_o = Reaction time without enzyme (constant)
t = Reaction time with enzyme
C = Conc. of extract (mg/ml)
V = Vol of extract which gave value "t"

Linear relationship between $(t_o - t)/t$ and concentration of enzyme was confirmed as shown in Fig. 1. In determination of enzyme activity, the value "t_o" was kept constant in 60 to 90 seconds, and the value "t" was adjusted to get "t" in the range of 10 to 40 seconds, whereby a linear relationship was obtained between $(t_o - t)/t$ and concentration of enzyme. The data obtained according to the above formula were approximately 3 times higher than those obtained by the Keil'n-Mann formula(6) used previously(3,4). Measurements of degree of pseudopregnant proliferation in the uterine endometrium were made as previously described(3,7) using the ratio of glandular (G) to total mucosal area (M).

Results. Table I presents measurements of carbonic anhydrase content and degree of pseudopregnant proliferation (G/M) in control rabbits receiving the vehicle alone and in rabbits injected with the 4 estrogens listed. It is clear from the data that there is no significant difference in mean carbonic anhydrase content or in mean G/M ratio in the control and treated groups. The dose/rabbit

TABLE I. Effect of Estrogen on Carbonic Anhydrase Activity and G/M Ratio of Clauberg Rabbits' Endometrium.

Compound	Dose (mg per rabbit)	No. of rabbits	EU/g wet tissue (Mean ± S.E.)	G/M ratio (Mean ± S.E.)
Control	Sesame oil	17	23 ± 3	.234 ± .019
Estrone	.25	5	"	.231 ± .016
Estradiol	"	5	22 ± 2	.226 ± .014
Estriol	"	5	27 ± 2	.233 ± .010
Stilbestrol	"	5	26 ± 3	.238 ± .013

is the maximum employed in antagonism experiments (*vide infra*) and suggests no direct effect of estrogens certainly up to this dose.

Table II contains data on endometrial response to varying dosages of progesterone and the effect of simultaneous constant dose of estradiol-17β at those pregesterone dosages which alone significantly increase the endometrial carbonic anhydrase content over the control. This estrogen dosage significantly inhibits stimulation both of carbonic anhydrase and of proliferation over the range 0.5 to 8.0 mg of progesterone. An S-shaped dosage-response curve is obtained in both sets of data, but that obtained with added estrogen is uniformly below that of progesterone alone.

Using a maximal stimulating dose of progesterone, 2 mg/rabbit, the effects of varying concentrations of the 4 estrogens were determined. As can be seen in Table III and Fig. 2, a characteristic dosage:response effect is had with each estrogen; statistical analysis of relationships exhibited in Fig. 2 establish linearity and parallelism of curves at the 1% level of probability.

Percentage inhibition of carbonic anhydrase and pseudopregnant proliferation effects are presented in Table IV. As is obvious also from Fig. 2, entirely similar degrees of inhibition are exhibited by all 4 estrogens on the carbonic anhydrase concentration. They exhibit effects parallel to each other on the G/M ratio, but dose for dose, G/M inhibition is less than carbonic anhydrase inhibition.

TABLE II. Dose-Response Relationships of Progesterone with and without Estradiol for Carbonic Anhydrase Activity and G/M Ratio of Clauberg Rabbits' Endometrium.

Dose of Progesterone (mg/rabbit)	Dose of Estradiol (mg/rabbit)	No. of rabbits	EU/g wet tissue (Mean ± S.E.)	G/M of uterus (Mean ± S.E.)
0	0	17	23 ± 3	.234 ± .019
.125	0	6	34 ± 8	.327 ± .028
.25	0	7	43 ± 5	.419 ± .031
.5	0	6	367 ± 18	.550 ± .047
1.0	0	7	415 ± 34	.661 ± .026
2.0	0	7	456 ± 44	.718 ± .018
8.0	0	7	401 ± 33	.720 ± .024
.25	.025	8	38 ± 2	.411 ± .020
.5	"	7	92 ± 18	.447 ± .037
1.0	"	6	121 ± 17	.499 ± .019
2.0	"	7	255 ± 51	.658 ± .023
8.0	"	9	235 ± 46	.647 ± .013

TABLE III. Inhibitory Effect of Estrogen on Progesterone in Carbonic Anhydrase Activity and G/M Ratio of Clauberg Rabbits' Endometrium.

Dose of progesterone (mg/rabbit)	Treatment of estrogen	No. of rabbits	EU/g wet tissue (Mean ± S.E.)	G/M ratio (Mean ± S.E.)
2.0	Control (Sesame oil)	18	492 ± 18	.732 ± .013
2.0	Estrone			
	.0025	6	345 ± 22	.626 ± .031
	.025	5	194 ± 16	.628 ± .022
	.25	5	108 ± 13	.467 ± .049
2.0	Estradiol			
	.0025	5	388 ± 34	.629 ± .024
	.025	5	281 ± 23	.658 ± .023
	.25	5	141 ± 13	.447 ± .039
2.0	Estriol			
	.0025	6	387 ± 26	.665 ± .018
	.025	6	304 ± 26	.675 ± .016
	.25	5	75 ± 9	.459 ± .038
2.0	Stilbestrol			
	.0025	6	400 ± 27	.657 ± .015
	.025	5	158 ± 14	.559 ± .059
	.25	6	67 ± 7	.384 ± .032

Discussion. Inactivity of estrogens by themselves and their quantitative inhibition of progesterone-stimulated endometrial carbonic anhydrase and pseudopregnant proliferation are clearly demonstrated by the foregoing data. Furthermore, linear relationship between log dose of estrogens and degree of reduction of the enzyme content in maximally stimulated uteri indicate quantitative usefulness of the enzyme test for assay of antiprogestational activity.

In the study of the effect of stilbestrol on rabbit endometrial carbonic anhydrase increases stimulated by progesterone (2 mg), 11-deoxycorticosterone (16 mg), 17-hydoxy-

TABLE IV. Percent Inhibition of Progesterone (2 mg) by Estrogen.

Compound	Dose (mg/rabbit)	% inhibition of progesterone (2 mg) Enzyme test	G/M test
Estrone	.0025	31	21
	.025	64	21
	.25	82	53
Estradiol	.0025	33	21
	.025	53	15
	.25	82	72
Estriol	.0025	22	14
	.025	40	11
	.25	89	55
Stilbestrol	.0025	20	15
	.025	71	35
	.25	91	70

% inhibition was calculated from data in previous table according to the following formula:

$$\% \text{ inhibition} = \frac{Po - Pe}{Po - C} \times 100.$$

Po = Response to progesterone. Pe = Response to progesterone along with estrogen. C = Control level.

progesterone-17 caproate (2 mg) Lutwak-Mann and Adams(8) observed a reduction in carbonic anhydrase when gestagens were administered in a single dose with 2 mg of stilbestrol. They noted also a reduction of pseudopregnant proliferation on an arbitrary grading scale. The superior sensitivity of a multi-dose procedure is obvious from our data. They noted also a somewhat lesser degree of differentiation on the basis of histological grading by the fifth day after progestin-estrogen administration. The sharper differentiation seen in the carbonic anhydrase determinations may be related to the wider range of response given by these measures over the G/M measures, as previously noted (4); alternatively, those processes leading to tissue proliferation are somewhat less sensitive to estrogen than those leading to carbonic anhydrase synthesis.

Estrogen-progesterone antagonism takes place in the endometrium as Courrier(1) demonstrated by local administration of estradiol into the closed lumens of uteri of progesterone-treated rabbits; this was confirmed by Heath *et al.*(9) by local administration of both hormones to rabbits as well as by combinations of local and systemic administrations of each hormone. However, another possible site of antagonism, such as neutralization or inactivation of progesterone in blood or in some tissue other than endometrium, can not be precluded by their experiments.

Comparing log-dose response curve of progesterone alone with that of progesterone in combination with estradiol, it is clearly observed that estradiol decreases maximal response of progesterone and makes the characteristic log-dose-response curve of progesterone flatter without changing the position of the curve. This is true in both enzyme determination and in the G/M ratio measurement. These results suggest that it is unreasonable to explain the estrogen-progesterone antagonism by neutralization or inactivation of progesterone by presence of estrogen in the body or by competition of both hormones for the receptor of the endometrium.

Adding our results to those of Courrier(1) and Heath *et al.*(9), it is reasonable to say that estrogen may depress reactivity of endometrium to progesterone by blocking somehow the process of progestational response in the endometrium.

In view of the widely differing estrogenic activities of these 4 estrogens on subcutaneous administration(10), their qualitative and quantitative similarities as progesterone antagonists are rather surprising. Presuming equal rates of absorption and concentration into the site of their action we may deduce that a rather specific aspect of their estrogenicity (or chemical structure) is involved. We plan to examine other diversely active estrogens or non-estrogenic derivatives to determine the extent and possible source of this inhibition.

Summary. 1) The anti-progestational activity of 4 estrogens—estrone, estradiol, estriol, and stilbestrol—administered subcutaneously along with progesterone into Clauberg rabbits has been demonstrated by estimation of endometrial carbonic anhydrase content and simultaneous measurement of uterine G/M ratio. 2) All these estrogens inhibit the effect of progesterone on carbonic anhydrase content and G/M ratio of the endometrium. Intensity of inhibition depends upon dosage. 3) The anti-progestational activities of these

estrogens are approximately the same. 4) When administered alone, these estrogens produce no significant change in either carbonic anhydrase titers or G/M ratios of the endometrium. 5) The linear relationship with negative slope which is obtained between the logarithmic dose of the estrogens and the endometrial carbonic anhydrase content of the progesterone-treated Clauberg rabbit suggests the usefulness of the carbonic anhydrase method as an assay procedure for anti-progestational activity. 6) Comparison of the log-dose-response curves of progesterone with and without estradiol indicates that estrogen may depress reactivity of the endometrium to progesterone rather than neutralize or inactivate progesterone in the body.

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Acid Hydrolysis of Erythropoietin.* (24391)

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We have recently isolated a heat stable erythropoietic factor, erythropoietin, and have shown it to be an acidic glycoprotein(1). Because several workers have suggested that erythropoietin is not a protein (2,3,4) it was of interest to investigate the possibility that this glycoprotein subserves a carrier function. Since dialysis of erythropoietin does not reduce its activity(5), any 'carried factor' would be firmly bound. Therefore, erythropoietin was submitted to acid hydrolysis and the degradation products studied biologically and qualitatively by chromatography.

Materials and methods. Purified erythropoietin was isolated from acidified, boiled plasma filtrates of anemic rabbits by DEAE-cellulose ion-exchange chromatography as previously described(1). It was dissolved in distilled water, 3 mg/ml. Five 1 ml aliquots were hydrolyzed in a water bath at 80° C for 1 hour with 5 ml H_2SO_4 in final concentrations of 0.005 N, 0.01 N, 0.04 N, 0.08 N and 0.10 N respectively. A 1 ml aliquot brought to 5 ml with distilled water was carried through the procedure as a control. The hydrolysates were separately dialyzed in the cold against repeated changes of distilled water for 5 days. The dialysates, concentrated in vacuo, and protein dialysands were lyophilized and stored. Separations of 1 mg of the hydrolyzed protein were carried out on Whatman 3 MM paper at 5 ma using veronal buffer pH 8.6, ionic strength 0.075. The strips were stained with bromphenol blue. Seven hour descending chromatograms of the dialysates in an ethyl acetate:pyridine:H_2O (10:4:3) solvent system(6) were prepared. A mixture of neuraminic (sialic) acid, d-glucosamine, d-mannose, d-glucose, d-galactose and l-fucose served as a reference material. After development and drying, the chromatograms were sprayed with aniline hydrogen phthalate reagent(7) and then heated at 110° C for 15 min. One half mg, in 0.9% NaCl, respectively of dialyzed 0.01 N hydrolysate of erythropoietin and in tact erythropoietin were injected subcutane-

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