tion or of storage blockade of tissue catecholamines.

Summary. Large doses of reserpine administered to pregnant rats on the ninth or tenth days of gestation are highly teratogenic. Pretreatment with reserpine significantly increases salicylate teratogenicity and augments the effect of immobilization on salicylate teratogenicity. A low, but significant teratogenicity also results from epinephrine administration on the tenth day. The types of anomaly produced by all these agents are similar except that reserpine or epinephrine also produce the anomaly of stunted fetuses lacking most of the axial skeleton. These findings suggest that disturbed catecholamine metabolism may be an important teratogenic factor.

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Some Biological Properties of a Urinary Norethynodrel Metabolite.* (29991)

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Some biological properties of a urinary metabolite of norethynodrel. The biological properties of norethynodrel and norethindrone have been extensively investigated(1, 2). This laboratory has been interested in the chemical nature and biological properties of metabolites of norethynodrel and norethindrone. The estrogenicity of norethynodrel has been reported to be 3-7% that of estrone (2). The extent to which this estrogenicity is due to aromatization to phenolic estrogens has been a subject of discussion.

Paulsen *et al*(3) bioassayed urines of patients receiving both norethindrone and norethynodrel and observed high estrogenic potency which they claimed to be due to the metabolites of the parent substances and not to the presence of ethynyl estradiol-3-methyl ether in the administered compounds. The present communication presents some biologi-

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TABLE I. Effect of Subcutaneously Injected Estrone and 17α-Ethynyl-19-Norandrost-4-ene-3β,10β, 17β Triol on Uterine Weight, Protein, and Carbonic Anhydrase of Immature Mice.

Treatment						Carbonic anhydrase	
Estrone (μg)	Test cmpd (μg)	No. exp	No. mice	Uterine wt (mg) mean ± S.E.	Protein per uterus (mg)	No. samples	E.U./g tissue mean ± S.E.
0	0	6	70	7.4 ± .44	.94	17	263 ± 18.1
0	10	1	10	9.0 ± .53	—	—	—
0	20	2	20	10.2 ± 1.16	1.38	4	258 ± 19.1
0	40	2	29	12.2 ± .55	1.59	12	385 ± 27.2
0	80	1	20	24.4 ± 1.37	2.98	5	958 ± 103.1
0	200	1	10	24.3 ± 1.40	2.78	5	970 ± 93.1
.05	0	2	18	16.1 ± 1.78	2.00	10	580 ± 78.5
.05	20	1	10	17.7 ± 1.07	2.27	5	588 ± 72.5
.05	40	1	9	24.0 ± 1.27	2.72	5	870 ± 74.4
.10	0	6	79	26.0 ± 1.99	3.01	35	881 ± 64.7
.10	10	1	9	28.5 ± 2.19	3.32	5	816 ± 80.2
.10	20	3	30	34.3 ± 1.93	3.83	15	1225 ± 67.1
.10	40	3	39	37.8 ± 2.53	4.13	20	1469 ± 117.1
.10	80	1	20	41.0 ± 1.79	4.55	5	1451 ± 92.6
.10	200	1	10	43.2 ± 2.21	4.60	5	1278 ± 51.1

cal properties of 17α-ethynyl-19-norandrost--4-ene-3β,10β,17β-triol, one of the major urinary metabolites of norethynodrel.

Materials and methods. Some of the 17α-ethynyl-19-norandrost-4-ene-3β,10β,17β-triol used in this study was prepared in this laboratory by the reduction of 17α-ethynyl-10β-hydroxy-19-nortestosterone(4). The remainder was a sample obtained from G. D. Searle and Co., through the courtesy of Dr. F. B. Colton. Both samples were crystallized twice from aqueous acetone. The purified material was chromatographically homogeneous(5). The compound melted at 110-115°, resolidified and melted again at 172-175°C.

Estrone and the test compound were dissolved in sesame oil and were administered to 21-day-old mice for 3 days. Autopsies were performed on the fourth day. Estrone was always injected, whereas the test compound was either injected or administered by gavage. When both compounds were given by injection, they were injected together in a total volume of 0.1 ml/day. Edgren *et al* (6) have shown that there is no advantage to separate site injections, and we felt that a single injection was less stressful than 2 injections per day. The following end points were studied: 1) uterine weight, 2) uterine protein content, 3) carbonic anhydrase (CA) activity. Uterine weights were determined by weighing the freshly excised tissue on a Roller-Smith torsion balance. Protein content was determined by a modified Folin phenol reagent method(7) and CA activity by manometric method(8).

Results and discussion. Table I contains data obtained from injections of estrone and of 17α-ethynyl-19-norandrost-4-ene-3β,10β, 17β-triol into immature mice. In view of the fact that only a limited number of dosage combinations could be examined during a single test period, there are more animals in certain groups than others. Protein analyses were conducted on the uterine homogenates utilized for CA determination and the number of individual analyses corresponds to the number of CA samples. Statistical analyses were carried out on each individual experiment. From Table I we have excluded results obtained with 0.2 and 0.4 μg of estrone because in our hands these dosages produced maximal stimulations without any additive or antagonistic effect by the test compound. The compound exhibited weak estrogenic activity without a linear dosage-response relationship.

Table II presents data on oral administration of the test compound alone, or with injected estrone. These data are from a single experiment. The estrogenicity of the test compound was considerably enhanced when administration was by the oral route (Table II). This suggests the possibility of conversion of the material to a more potent estrogen. Aromatization of 3-keto-19-norsteroids by acid has been observed by several workers (4,9). Estrogenicity of norethynodrel is not

TABLE II. Effect of Estrone (injected) and 17α-Ethynyl-19-Norandrost-4-ene-3β,10β,17β Triol (gavaged) on Uterine Weight, Protein, and Carbonic Anhydrase of Immature Mice.

Treatment					Carbonic anhydrase	
Estrone (μg)	Test cmpd (μg)	No. mice	Uterine wt (mg) mean ± S.E.	Protein per uterus (mg)	No. samples	E.U./g tissue mean ± S.E.
0	0	25	6.6 ± .21	.82	4	238 ± 26.2
0	10	10	14.3 ± 1.10	1.47	5	447 ± 36.6
0	20	10	18.4 ± 1.20	2.19	5	839 ± 81.1
0	40	10	26.8 ± 2.08	3.14	5	1447 ± 71.5
.1	0	10	25.8 ± 2.04	3.05	5	994 ± 19.7
.1	20	10	35.6 ± 1.86	3.82	5	1365 ± 33.8
.1	40	10	35.1 ± 2.78	3.76	5	1347 ± 78.7
.4	0	10	47.4 ± 3.11	5.32	5	2285 ± 126.6
.4	10	10	42.2 ± 2.50	4.41	5	2291 ± 166.3
.4	20	10	46.5 ± 3.05	5.11	5	2437 ± 117.5
.4	40	10	43.5 ± 2.10	4.83	5	2143 ± 253.8

enhanced by the oral route of administration, and 10 to 40 μg of norethynodrel results in mouse uterine weights of approximately 40 mg irrespective of the route.

Examination of Table I reveals that combination of 0.1 μg of estrone with 20 or 40 μg of the test compound resulted in uterine weights and CA activities greater than would be expected from a simple additive effect of the 2 compounds. Estrone at the dose of 0.1 μg resulted in increment of uterine weight of 18.6 mg. The test compound doses of 10, 20, and 40 μg resulted in increases of 1.6, 2.8, and 4.8 mg respectively. Simple additive effect of combined injections would result in increments of 20.2, 21.4, and 23.4 mg. However, actual uterine weight increases were 21.1, 26.9, and 30.4 mg respectively (Table I). The synergistic effect observed from the combination of 0.1 μg of estrone and 20 and 40 μg of the test compound was statistically significant ($P<0.05$). Examination of CA values likewise indicates synergism between the two compounds.

No synergism or simple additive effect was observed when the test compound was administered by gavage (Table II). Uterine weights resulting from the combination of 0.1 μg estrone with 20 and 40 μg of the test compound were significantly heavier ($P<0.01$) than the weights resulting from single treatments. The failure to observe synergism may reflect the enhanced estrogenicity resulting from gavage as opposed to subcutaneous administration of the metabolite. Although full additive effects were not observed, there was no evidence of true antiestrogenicity of the compound at the dose levels studied.

Many examples of antagonism between weak estrogens and potent ones are known (10,11), but potentiation of the effect of estrogen in the mouse uterine weight and CA assays have not been reported. The dose levels used in the present work may be an important factor in the results, but nonetheless the potentiation of estrogenic activity might be a specific biological effect of the test compound. The only report dealing with enhancement of estrogenic activity is that of Dorfman *et al*(12) who have published evidence for enhancement of estrogen activity by a male urine fraction. However, the chemical nature of the enhancer was unknown.

The high degree of biological estrogenicity observed in extracts of urine from patients receiving norethynodrel(3) may be at least partly explained if the estrogenic effect of other compounds in the urine were potentiated by 10-hydroxylated metabolites of norethynodrel. In the case of norethynodrel, it has been shown that 10β-hydroxy derivatives, including the material reported here, comprise a large percentage of the metabolites excreted in urine(13,14).

Summary. Estrogenic activity of 17α-ethynyl-19-norandrost-4-ene-3β,10β,17β-triol, a urinary metabolite of norethynodrel, was investigated. The compound exhibited slight estrogenicity when administered subcutaneously. Gavage resulted in increased estrogenicity. Within a limited dose range, injection of the compound together with estrone resulted in synergistic estrogenic activity.

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Alteration of RES Phagocytic Function by Pyrogen Contaminated Colloidal Carbon.* (29992)

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Various colloidal preparations have been used to study the phagocytic function of the reticuloendothelial system (RES) often with variable and conflicting results. One prerequisite for such studies is that the colloid be non-toxic for RE-cells and the animal(1). Halpern *et al*(2) found that the toxic component of India ink was an organic shellac. They subsequently obtained a special preparation of India ink in which "fish glue" was substituted for shellac. This special ink ("Special Biological Ink" C11 1431a) is available commercially and is widely used in studies of RES phagocytic function. From the literature concerning pyrogenic therapy, it is apparent that proteinaceous substances free of pyrogen are exceptional. Seibert's work(3) is outstanding in showing that pyrogens are almost exclusively products of Gram-negative bacteria.

In preliminary studies we observed considerable pyrogenic response in rabbits after intravenous injection of the special biological ink. This report gives the results of further studies relating to this observation.

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Materials and methods. Colloidal carbon. "Special Biological Ink": C11 1431a (Gunther Wagner Co., Hannover, Germany) was obtained from the John Henschel Co., New York. It was prepared in 1% gelatin (Difco, certified) to a carbon concentration of 16 mg/ml after the large aggregates had been removed by centrifugation at 2,930 *g* for 15 minutes. The dry weight of carbon remaining was 110 mg/ml. The carbon suspension was then autoclaved at 15 psi for 15 minutes and stored in sealed 10 ml glass vials at 4°C. It was warmed to 37°C and thoroughly mixed before use.

Endotoxin. The purified *Escherichia coli* 08 $C008_{2158}S_5$ endotoxin was obtained from Dr. Otto Westphal, Max Planck Institute, Freiburg, West Germany. The dose used, 0.2 μg/kg, was equivalent to 25 MPD-3/kg (Minimal Pyrogenic Dose at 3 hours)(4).

Test animals. American-Dutch rabbits were obtained from a single supplier. They were housed in air-conditioned quarters and the clearance tests were performed in a similar environment. Adult animals were selected to weigh 1.0 to 1.5 kg. Young animals weighed on the average 0.3 kg.

Cortisone treatment of rabbits. A group