Effects of Orally Administered Progesterone-Like Compounds on Mammary Gland Growth in Rats.* (28377)

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When administered orally several of the synthetic progesterone-like compounds have been effective in producing one or more of the physiological effects of progesterone. The determination of desoxyribonucleic acid (DNA) has been used extensively as a quantitative index of mammary gland growth under normal and experimental conditions. The present study was conducted to determine the effectiveness of several progesterone-like compounds administered orally and by injection at various levels to synergize with daily injections of 1 μg estradiol benzoate (E.B.) to grow the mammary gland of ovariectomized rats.

Materials and methods. Young adult virgin female rats of Sprague-Dawley-Rolfsmeyer strain were ovariectomized 10 days before starting experimental treatment. Rats were housed 5 to a cage in a room artificially illuminated during daylight hours and kept at a uniform temperature of 78 ± 1°F. Rats administered estrogen with oral progesterone-like compounds were injected with 1 μg E.B. daily throughout 19-day treatment period. Progesterone-like compounds, 17 α hydroxy-6 methylpregn-4 en-3 3,20-dione one 17 acetate (Provera‡), 17 α ethinyl-17-hydroxy 19-nor-androst-4-en-3-one (Norlutin‡), 17 α ethinyl-19 nor-androst-4 en-3-one 17-acetate (Norlutate‡), 6 chloro-6-dehydro-17-acetoxy-progesterone (Lutoral‡) were mixed in feed ground Purina Lab Chow) at a level in which the desired amount administered would be consumed in daily ration. Daily weigh-back of the feed was made to determine the amount of the progesterone-like hormone the rats received (Table I). Norlutin Enanthate‡ (17 α-ethinyl-19 nor-androst-4 en-3-one 17 enanthate) was injected at levels of 3 and 6 mg with 1 or 2 μg E.B. for 19 days in 4 groups of rats. Animals were sacrificed on day 20 and the 6 abdominal-inguinal mammary glands were removed for DNA determination as described by Griffith and Turner(1).

Results. Mammary gland proliferation stimulated by Norlutin or Norlutate given orally plus E.B. by injection showed DNA values not significantly greater ($P<.05$) than the DNA values obtained with E.B. injected alone indicating that these compounds did not stimulate mammary gland growth in synergism with estrogen. Levels of Norlutin, 2.16 mg/day or Norlutate, 2.22 mg/day fed alone stimulated significantly less mammary gland growth than E.B. injected alone ($P<.02$ and $<.01$). The feeding of lower levels of Lutoral (.075 to .3 mg/day) and the injection of 1 μg E.B. stimulated mammary gland growth as measured by DNA greater than by E.B. alone. However, when Lutoral feeding was increased to 1 to 2.98 mg/day, the gland growth obtained did not differ significantly from the group receiving E.B. + P. Similar gland growth was observed by the feeding of Provera at levels of 1 to 5.2 mg/day.

The injection of 3 or 6 mg/day of Norlutin Enanthate with 1 or 2 μg E.B. stimulated gland growth, as measured by DNA, which was not significantly different ($P<.01$) from the growth of rats pregnant 18-20 days or treated with 1 μg E.B. + 3 mg P/day. In most of the animals on these compounds, body weight loss occurred (Table I).

Discussion. This study was conducted to find orally effective progesterone-like com-

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2551. Approved by Director. This investigation was supported by grants from USPHS and Am. Cancer Soc.

† Public Health Service Research Fellow of Nat. Cancer Inst.

‡ Norlutin, Norlutate, and Norlutin Enanthate kindly supplied by Parke, Davis & Co., Lutoral by Syntex Corp., and Provera by Upjohn Co.

TABLE I. Experimental Growth of Mammary Gland in Ovariectomized Rats.

Treatment	No. of animals	Initial body wt (g) mean	Final body wt (g) mean	DFFT* (mg) mean	DNA (μg/mg DFFT)	Total DNA (mg/100 g body wt)
18-20 day pregnancy	19	253	324	749	33.2 ± .8	7.69 ± .4
1 μg E.B. +3 mg P—19 days	21	273	278	606	31.5 ± 1.0	6.81 ± .4
2 " " +6 " " "	22	296	292	606	29.3 ± 1.5	6.06 ± .4
1 " "	20	235	250	340	25.4 ± 1.5	3.30 ± .3†
Orally						
1 μg E.B. + .14 mg Norlutin	10	275	242	413	21.0 ± 1.0	3.70 ± .4†
" " " + .28 " "	10	265	216	392	23.5 ± 1.4	3.94 ± .3†
" " " + .46 " "	10	265	222	341	24.6 ± 1.0	3.83 ± .3†
" " " +2.8 " "	10	245	237	328	27.4 ± 1.5	3.74 ± .3†
" " " + .9 " Norlutate	10	224	203	307	25.3 ± 1.4	3.73 ± .5†
" " " +2.7 " "	8	317	244	315	27.8 ± .7	3.90 ± .3†
Norlutin 1 mg	5	212	225	353	17.2 ± .4	2.70 ± .1†
" 2.16 "	5	220	209	225	20.4 ± 1.3	2.19 ± .1†
Norlutate .9 "	5	221	220	341	16.3 ± .4	2.49 ± .0†
" 2.22 "	5	230	229	267	18.2 ± .9	2.10 ± .1†
1 μg E.B. + .075 mg Lutoral	10	308	291	519	23.8 ± .9	4.26 ± .5†
" " " + .15 " "	10	306	292	542	27.2 ± 2.2	4.78 ± .6†
" " " + .3 " "	10	306	288	532	24.3 ± 1.3	4.59 ± .6†
" " " +1.0 " "	10	234	255	478	35.3 ± 1.5	6.60 ± .3
" " " +2.98 " "	7	292	284	638	28.1 ± 1.2	6.19 ± .3
" " " +1.0 " Provera	10	285	277	598	33.7 ± 2.8	7.10 ± .7
" " " +3.0 " "	10	271	265	611	29.8 ± 1.2	7.00 ± .3
" " " +5.2 " "	10	266	268	656	31.3 ± 1.6	7.70 ± .3
Injection						
1 μg E.B. +3.0 mg Norlutin enanthate	12	275	236	522	38.7 ± 1.0	7.24 ± .5
" " " +6.0 " "	12	256	237	473	41.3 ± .9	6.83 ± .2
2 " " +3.0 " "	8	255	233	411	32.4 ± 1.2	6.94 ± .5
" " " +6.0 " "	10	261	251	517	28.1 ± 1.4	7.10 ± .4

* Dry fat free tissue.
† Significantly less at the 1% level when compared to the 1 μg E.B. +3 mg P treatment.

pounds capable of synergizing with estradiol benzoate to stimulate lobule-alveolar growth in the rat. The most effective dosage level of Lutoral or Provera was found to be 1 mg/day. Difficulty was observed in obtaining consumption of higher levels of the hormones; however, one group consumed 5.2 mg Provera/day and made a slight gain (2.8 g/rat) in body weight during the experiment.

Using total DNA as an index of growth, daily injections of 1 μg E.B. and 3 mg progesterone (P) in ovariectomized rats for 19 days resulted in mammary gland growth comparable to that of rats pregnant 18-20 days(2). Data presented here indicate that Lutoral or Provera fed at the 1 mg level/day were quite comparable to the injection of 3 mg P in synergizing with 1 μg E.B. to stimulate mammary gland proliferation. While lower levels of these substances were less effective, higher levels of administration did not result in significantly higher DNA values.

The modified testosterone compounds Norlutin and Norlutate were ineffective in stimulating mammary gland development either when fed alone or with daily injections of 1 μg E.B. at a dosage level of 2.8 mg/day or less. Norlutin Enanthate proved to be as effective as progesterone when injected with E.B. The injection of 3 and 6 mg Norlutin enanthate with 1 or 2 μg E.B. daily produced DNA values equal to those obtained with 1 μg E.B. + 3 mg P daily for 19 days in ovariectomized rats.

The losses of body weight in many rats fed the progesterone-like compounds in this study were considerable. The preliminary work indicated these substances were unpalatable to rats. Various concentrations of sugar, syrups and egg albumin used to flavor the feed, plus providing a binding agent for the purpose of repelleting the feed,

failed to improve the consumption of these hormones in the feed. The 4 groups of rats injected with Norlutin Enanthate declined similarly in body weight to those fed the other 4 compounds. Gains in body weight were observed in only 3 of the 22 groups of experimental animals.

Summary. The oral effectiveness of 4 progesterone-like compounds to stimulate mammary gland growth (DNA) of ovariectomized rats is reported. Norlutin and Norlutate plus E.B. were ineffective in stimulating increasing DNA in comparison with E.B. alone. Lutoral and Provera plus E.B. at the 1 mg level or above stimulated comparable mammary gland growth to that observed at end of pregnancy or after 1 μg E.B. + 3 mg P. The injection of 1 or 2 μg E.B. plus 3 or 6 mg Norlutin Enanthate was also effective in stimulating DNA values comparable to normal pregnancy. The losses in body weight of most groups during the 19-day feeding period were apparently due to the unpalatable nature of feed containing these compounds.

1. Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 448.

2. Moon, R. C., Griffith, D. R., Turner, C. W., *ibid.*, 1959, v101, 788.

Received March 14, 1963. P.S.E.B.M., 1963, v113.

Tissue Culture Cytopathic and Plaque Assays for Mouse Hepatitis Viruses. (28378)

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Although viruses of the mouse hepatitis (MHV) group have been propagated in a variety of tissue cultures(1-8), none of the systems described has been shown to be of broad applicability. In most instances the virus under study did not induce cytopathic changes or did so only after adaptation; the systems in which cytopathic changes occurred were not useful for more than one or two of the laboratory passaged strains, and were insensitive compared to animal inoculation. Manaker *et al.*(5) reported a cytopathic assay in a continuous cell line of C3H mouse liver, clone NCTC 1469(9), for 2 strains, the A-59(5) and MHV-2(10) viruses; however, they did not find this cell line suitable for assay of the MHV-1(11) and H747(12) strains. With slight modifications of Manaker's procedures, principally by substitution of chicken serum-containing maintenance media, we have found the NCTC 1469 line to be useful for assays of all strains of MHV studied, including the various prototype laboratory strains, freshly isolated field strains, and to a limited extent, virus in field specimens, and to be suitable for plaque assays of the great majority of the strains.

Materials and methods. Seed stocks of NCTC 1469 were received from Dr. Robert A. Manaker and Dr. Virginia J. Evans, Natl. Cancer Inst.; the 2 sublines reacted identically, and the latter was used for routine work.* Cell stocks were grown in 2 oz or 32 oz bottles, in a medium consisting of 20% horse serum in NCTC 109(13). Passages were made at 7 day intervals by vigorously shaking the cells into the fluid; passage by scraping cells off the wall yielded cultures which were resistant to infection.

Tube cultures were planted with 4×10^5 cells in 1 ml of the growth medium; 35 mm diameter plastic Petri dishes (Tissue Culture Dish, Falcon Plastics, Los Angeles) with 6×10^5 cells in 1.5 ml; and 60 mm dishes with 1.4×10^6 cells in 3.5 ml. Tube and bottle cultures were tightly stoppered, and Petri dish cultures were kept in a humidified

* The majority of cultures used were produced on contract by Microbiological Associates Inc., Bethesda, Md.