sera as described by Chang(1,11) has not been a problem. We have found that only one of 23 human serum pools was toxic to FL cells.

In preliminary experiments with FL and HeLa cells the FL cells have not produced tumors in treated rats using a technic‡ in which HeLa cells produced significant tumors. More work is needed however before a conclusion can be established.

Summary. A strain of human cells derived from a normal amniotic membrane has been cultivated in serial passage for 8 months in 30 transfers. This cell line was developed by trypsinization of the primary source and cultivated in a medium without embryo extract. The appearance of the cell has been epithelial-like at all stages of cultivation. Growth characteristics in media containing human serum or animal sera are reported. The cell strain has been grown in large scale.

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Received December 19, 1956. P.S.E.B.M., 1957, v94.

Estrogen Antagonisms: Inhibition of Estrone-Induced Uterine Growth by Testosterone Propionate, Progesterone and 17-ethyl-19-nortestosterone. (23004)

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Recent literature has demonstrated a considerable interest in substances which antagonize the action of estrogenic compounds. Androgens have long been known to inhibit, at least in part, the effects of estrogens on various target organs(1). Progesterone, corticoids and various estrogenic substances also have been implicated (see 2 for review of literature). The reports generally leave much to be desired from a comparative standpoint as end points used vary from laboratory to laboratory and few workers have attempted more than qualitative analyses; thus adequate potency evaluations are difficult. The present communication discusses an estrogen-antagonistic action of 17-ethyl-19-nortestosterone (Nilevar) and compares activity of this substance to activities of testosterone propionate and progesterone, using estrogen-induced uterine growth as an index of activity.

Materials and methods. The estrone-stimulated uterine growth of intact, immature mice was employed for purposes of assay following essentially the procedure of Rubin, *et al.*(3). Our modification of this test has already been described(4). The response index was weight of uterus in mg. Five experiments were carried out using each of the 3 substances: progesterone, testosterone propionate and 17-ethyl-19-nortestosterone. A dose of 0.3 μg of estrone was employed as standard uterine growth-stimulator, against which the test compounds were assayed. In each experiment groups of 8-10 mice were treated with the estrone standard alone and estrone in combination with a series of doses

of test substance; an additional group of mice served as control receiving only corn oil injections. In analyses of data, it was assumed that a 100% inhibitor, when administered simultaneously with 0.3 μg of estrone, would produce a uterine weight equivalent to that of simultaneously run oil-treated controls. Thus for each experiment the difference between response obtained with 0.3 μg of estrone and that obtained with oil treatment was considered a 100% depression. Uterine weights of groups receiving inhibitor were compared to this total possible depression and percentage value obtained. In a typical test uteri of estrone-treated mice averaged 38.5 mg while oil control uteri averaged 9.8 mg; the difference, 28.7 mg, was considered 100%. A combined dose of 0.3 μg estrone plus 2 μg of 17-ethyl-19-nortestosterone, which gave an average response of 24.2 mg, was considered a 49.8% depression. Each experiment was calculated separately. These percentage depressions and logs of doses of antagonist were fitted with dose response lines using the method of least squares.

Results. All 3 substances produced marked depression of the estrone-induced uterine growth response (Fig. 1). Although the responses, as may be expected, were highly variable, the data supported the contention that 17-ethyl-19-nortestosterone was a potent inhibitor of estrone activity, being roughly 70 times as potent as testosterone propionate and about 50 times as potent as progesterone (Table I). Progesterone appeared to be slightly more active than testosterone propionate (about 1.4 times). Statistical analysis indicated adequate parallelness among the 3 slopes, so a single pooled value was used.

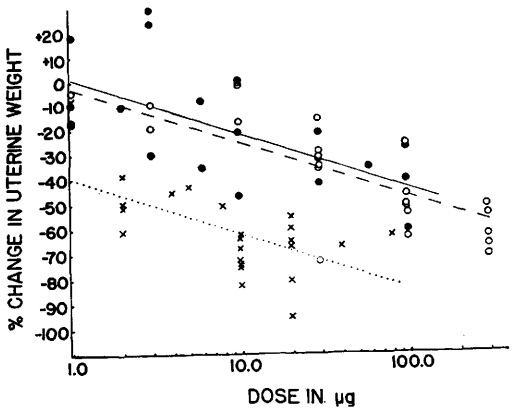

FIG. 1. Effects of testosterone propionate (dots and solid line), progesterone (circles and dashed line) and 17-ethyl-19-nortestosterone (crosses and dotted line) on estrone-induced uterine growth of intact, immature mice. 0% change = response of uterus to 0.3 μg of estrone; −100% change = control uterine level.

TABLE I. Regression Formulae of Uterine Growth Depression on Dose of Estrogen-Antagonist, and Potencies of Compounds Relative to Testosterone Propionate. y = % depression of uterine wt; x = log dose of antagonist. For definition of depression see text.

Antagonist	Regression formula	Relative potency
Testosterone propionate	y = 1.04 − 22.20x*	1.00
Progesterone	y = − 2.28 − "	1.41
17-ethyl-19-nortestosterone	y = −39.98 − "	70.42

* b = −22.20 ± 2.93.

Discussion. 17-ethyl-19-nortestosterone is a synthetic compound with many properties similar to natural steroids: it has androgenic and anabolic properties, progestational activity, metrotropic activity, and estrogen antagonistic action. Comparisons among these are of interest. Saunders and Drill(5) showed it to be as active as testosterone propionate as anabolic agent when measured by levator ani response whereas it had only about 6% androgenic activity. 17-ethyl-19-nortestosterone is a progestational agent; it was more active than progesterone in the Clauberg assay (5-10 times), but a somewhat higher dose was required to maintain pregnancy in spayed rabbits(6).

Data on androgenic, anabolic and progestational activities show marked lack of correlation with estrone-inhibitory action of the compounds; in most studies 17-ethyl-19-nortestosterone was less active than testosterone propionate or progesterone, the Clauberg assay being an exception. Even in the latter test, however, the potency was considerably less than the estrogen-antagonistic action reported here. Therefore, estrogen antagonism appears to be a unique pharmacological property, distinct from androgenic, anabolic or progestational actions. All 3 substances have

metrotropic activity as measured by uterine growth in immature mice*, however, these uterine-growth stimulating effects were not marked at doses below 100 μg where estrogen-inhibitory activities were quite apparent. Progesterone and 17-ethyl-19-nortestosterone appeared to be less potent than testosterone propionate in stimulating uterine growth, and all 3 have dose-response curves with shallow slopes similar to those of so-called impeded estrogens(4,7). It is of particular interest that a number of compounds which have dose-response curves with extremely shallow slopes will antagonize the action of estrone on the uterus. Preliminary studies on the effects of 17-ethyl-19-nortestosterone as an antagonist of estrone-induced vaginal cornification suggested that keratinization of epithelial elements proceeds in a relatively normal manner, but that leucocytes are never absent from the smears(8).

Summary. Testosterone propionate, progesterone and 17-ethyl-19-nortestosterone are all antagonists of estrone-induced uterine growth in intact immature mice. Progesterone has about 1.4 times and 17-ethyl-19-nortestosterone has about 70 times the potency of testosterone propionate. This high activity of 17-ethyl-19-nortestosterone did not appear to correlate with the androgenic, anabolic, progestational or metrotropic properties.

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Received December 26, 1956. P.S.E.B.M., 1957, v94.

Strains of Small Race *Entamoeba histolytica* Maintained in Outdated Blood Bank Blood Medium.* (23005)

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Interest has been growing in culturing strains of small race *Entamoeba histolytica,* especially since new strains can be isolated and maintained easily(1). Although strains of large race have been cultured in media containing whole or defibrinated blood or red blood cells from various sources(2-10), strains of small race have not been grown in whole human blood. Recently, Tarshis(11) prepared a blood medium for growing *Mycobacterium tuberculosis.* It is the purpose of the present paper to describe 2 modifications of this medium which have been adapted for the maintenance of strains of *E. histolytica,* particularly those of the small race.

Materials and methods. 1. *Diphasic medium.* Outdated blood bank blood approximately 4 weeks old was used and contained ACD solution as follows: citric acid U.S.P., 0.50 g, sodium citrate U.S.P., 1.37 g, and dextrose U.S.P., 2.45 g/100 ml of transfusion solution.† The medium was made as follows: For basal medium, heat to dissolve 6 g of agar-agar in 276 ml of distilled water containing 4 ml of glycerol. Autoclave at 15 lb pressure for 15 minutes. Store in icebox or use immediately after by cooling to 45°C in

* This work was supported in part by grant from N. I. of Allergy and Infectious Diseases, U.S.P.H.S.

† 480 ml of blood was collected in Baxter Transfuso-Vac bottles containing 120 ml of ACD solution.