# Long-Term Effect of Estrogen and Progesterone on Mammary Gland Growth in 3-Methylcholanthrene Treated and Non-treated Ovariectomized Rats.* (26487)

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Normal mammary gland growth and development of mammary gland carcinomas appear to be hormone dependent. Estradiol benzoate (EB) and progesterone (P), injected into rats in optimal levels, will produce mammary gland development (as determined by DNA estimation) equivalent to that produced during normal pregnancy(1). Further mammary gland growth may be obtained experimentally in the rat by concomitant administration of other hormones(2,3). Increased mammary gland growth has been observed after recurrent pregnancies in the mouse(4). Since above evidence indicated possibility of increased growth, it seemed desirable to determine effect of long term injection of optimal levels of EB and P on mammary gland growth in ovariectomized rat.

Rapidity of development of mammary gland carcinoma in rat by 3-methylcholanthrene has been shown to be dependent upon hormonal status of animal(5,6). Ovariectomized rats develop tumors much less rapidly or consistently than normal controls, while those treated with either estrogen or progesterone appear to develop carcinoma at a more rapid rate. Therefore, it was felt that effect of 3-methylcholanthrene on mammary gland growth produced by long term injection of optimal levels of EB plus P should be studied.

*Materials and methods.* Young, mature female albino rats of Sprague-Dawley-Rolfsmeyer strain were ovariectomized and fed a commercial laboratory animal feed. Two weeks after ovariectomy animals were divided into groups receiving 1 μg EB plus 3 mg P daily, 3-methylcholanthrene (3-me) alone, 3-me plus 1 μg EB and 3 mg P daily, and 3-me plus 2 μg EB plus 6 mg P daily. EB and P were dissolved in an olive oil carrier such that 0.1 ml solution was injected subcutaneously daily. Ten mg 3-me was administered orally by forced intubation twice weekly for a period of 7 weeks. This dose has been reported adequate for production of mammary gland carcinoma in approximately 50% of intact animals within this period(5). Groups of rats receiving EB plus P daily were sacrificed after 20, 30, 40 and 50 days. All groups receiving 3-me were sacrificed after 50 days. The 6 posterior mammary glands were removed and frozen. DNA was determined as described previously(1).

*Results.* Mean total DNA/100 g B.W. of rats receiving EB plus P for 20 days was significantly greater than ovariectomized rats receiving E alone (Table I). Although DNA values generally increased up to 40 days of injection, no significant differences were found between groups injected for varying lengths of time up to 50 days, indicating only slight additional growth is produced by long-term treatment with EB and P.

Mammary gland DNA of rats receiving 3-me alone was not significantly different from that of ovariectomized controls, indicating that this level of carcinogen was ineffective in production of mammary gland growth. DNA of mammary glands of rats receiving either 1 μg EB plus 3 mg P daily for 50 days plus 3-me, or 2 μg EB plus 6 mg P daily for 50 days plus 3-me was significantly depressed as compared to DNA of rats receiving only 1 μg EB plus 3 mg P for 50 days, indicating an inhibitory mechanism of action of 3-me. No mammary tumor formation was observed in these rats on gross visual inspection.

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TABLE I. Effects of Estradiol Benzoate (EB), Progesterone (P) and 3-Methylcholanthrene on DNA of Rat Mammary Glands.

| Treatment | No. of rats | Mean body wt, g | 6 posterior mammary glands: DFFT,* mg | DNA (μg)/ DFFT (mg) | Total DNA, mg | DNA/100 g BW, mg |
|---|---|---|---|---|---|---|
| 1 μg EB + 3 mg P 20 days | 15 | 221 | 430 | 37.5 | 16.13 | 7.3 ±.60‡ |
| *Idem* 30 " | 12 | 231 | 408 | 44.7 | 18.25 | 7.9 ±.42 |
| " 40 " | 10 | 246 | 396 | 52.8 | 20.91 | 8.5 ±.76 |
| " 50 " | 15 | 252 | 442 | 46.7 | 20.66 | 8.2 ±.55 |
| 3-methyl cholanthrene, 50 days | 12 | 270 | 243 | 36.6 | 8.91 | 3.3 ±.40 |
| 3-methyl cholanthrene, 1 μg EB + 3 mg P, 50 days | 12 | 266 | 362 | 49.2 | 17.82 | 6.7 ±.63 |
| 3-methyl cholanthrene, 2 μg EB + 6 mg P, 50 days | 12 | 272 | 375 | 44.96 | 16.86 | 6.2 ±.52 |
| Ovariectomized controls† | 15 | 273 | 359 | 23.20 | | 3.05±.14 |
| 1 μg EB, 19 days† | 5 | 246 | 485 | 22.86 | | 4.48±.15 |
| 1 μg EB + 3 mg P, 19 days† | 19 | 269 | 590.1 | 35.65 | | 7.72±.25 |

* Dry, fat-free tissue. † Data from Moon *et al.*(1). ‡ Stand. error of mean.

*Discussion.* Using DNA as an index of growth, it has been shown that a 1 to 3000 ratio of EB to P injected for 19 days produced growth equivalent to that observed in mammary glands of rats on 19th day of pregnancy(1). Recent evidence(7), however, indicated that approximately 40% of mammary gland growth occurs after parturition. Recurrent pregnancy has been shown to stimulate greater mammary gland growth in mouse(4), indicating that some form of hormonal stimulation over a longer period of time may be effective. Present study indicates that long term injection of constant levels of EB plus P is ineffectual in producing significantly greater growth than that produced during 19-20 days. However, it is significant that during 50 days injection period, DNA remained above level observed on 19th day of pregnancy, indicating that this hormonal treatment prevented involutionary changes. It would appear that other hormones, or other levels of these 2 steroids may be involved in production of greater growth than that observed during pregnancy. That greater growth may be produced over a 19 day period by synergism of thyroxine and growth hormone with above steroids has been shown(2,3).

It was noted that 3-me reduced mammary gland development by approximately 25% in animals receiving either 1 μg EB plus 3 mg P or double that amount.

Levels of 3-me used were observed to produce carcinogenic changes in intact animals (5). Present study indicates that levels of hormone injected in the 3-me treated ovariectomized animal prevented observable tumor development.

*Summary.* Administration of 1 μg estradiol benzoate plus 3 mg progesterone into ovariectomized rats for 20 days produced mammary gland DNA levels comparable to those observed in rats on 19th day of pregnancy, while continued injection for periods of 30, 40 and 50 days did not significantly increase observed growth. However, no involutionary changes were noted with long-term injection, indicating a maintainence of the lobule-alveolar system. Administration of 10 mg 3-methylcholanthrene twice weekly for 7 weeks reduced mammary gland growth by approximately 25% in ovariectomized rats given 1 μg estradiol benzoate plus 3 mg progesterone or twice this amount. These hormones prevented development of observable mammary tumors.

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## Reaction of Rheumatoid Sera with Fragments of Papain-Digested Rabbit Gamma Globulin.* (26488)

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Sera from a large proportion of patients suffering from rheumatoid arthritis react with rabbit gamma globulin as demonstrated by hemagglutination(1,2) and quantitative precipitin(3,4) technics. The reactant material in these sera has been termed "rheumatoid factor" and has the physicochemical properties of a gamma macroglobulin(5).

Porter(6) chromatographed a papain digest of rabbit gamma globulin on carboxymethylcellulose and obtained 3 fractions. These fragments (designated Fractions I, II, and III in the order of chromatographic elution) all had sedimentation constants of about 3.5S and molecular weights of about 50,000, 50,000, and 80,000, respectively(7). Fractions I and II were similar in chemical and biological properties. When antibody globulin was digested, each had the power to combine with, but not to precipitate, the homologous antigen. Fraction III showed no antibody activity but appeared to carry most of the antigenic determinants of the native molecule as determined by its ability to precipitate with antisera to rabbit gamma globulin.

In view of this localization of the antigenic determinants of rabbit gamma globulin on a fragment comprising about 40% of the intact molecule, it was of interest to determine which fragment or fragments of the papain digest were involved in reaction with rheumatoid factor. This would serve as a first step in comparing the structural units of the globulin molecule involved in reaction with rheumatoid factor and with specific antibody to rabbit gamma globulin.

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*Materials and methods.* γ-Globulin. Rabbit gamma globulin (RGG) was prepared by sodium sulfate precipitation according to the method of Kekwick(8). Paper electrophoresis of the preparation demonstrated a single component with the mobility of γ-globulin.

*Crystalline papain*† was prepared from crude enzyme powder and crystallized as the inactive mercury dimer(9). It was lyophilized and stored as the dimer.

*Sera.* A pool of sera from rheumatoid arthritis patients was kindly provided by Dr. Wallace V. Epstein, Dept. of Medicine, Univ. of California, San Francisco. The titer of this rheumatoid pool (RP) for tanned sheep erythrocytes coated with human γ-globulin (Cohn Fraction II) was 1:7000. In 3 experiments using tanned sheep RBC coated with RGG, the titer was found to be 1:896 to 1:1792.

Mice were immunized‡ with Fraction I of papain-digested RGG. The antigen was incorporated into a medium consisting of equal volumes of saline and Freund's adjuvant. Mice received 3 injections, each containing 1.0 mg of Fraction I. They were bled 10 days after last injection and the sera pooled.

Prior to use in experiments, all sera were incubated at 56°C for 30 minutes to inactivate complement and absorbed with an equal volume of washed packed sheep RBC for 18 hours at 4°C to remove heterophile antibody activity.

† Mercuripapain was prepared while the author was at the laboratory of Dr. R. R. Porter, Nat. Inst. for Medical Research, London, England.

‡ Mice were immunized and bled by Dr. Leon S. Kind, Dept. of Microbiology, Univ. of California, San Francisco.