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Suppression of Lactation by Tumor Promoters in Mice[1] (40993)

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Abstract. As a possible step to evaluate the role of tumor promoters in normal cells, the effects of phorbol and its ester, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), on normal mammary gland function and pituitary secretion of growth hormone (GH) and prolactin, were examined in lactating C3H/He mice. Daily subcutaneous injections of 400 μg phorbol or 4 μg TPA during Days 12 and 14 of lactation resulted in a significant decline in litter growth rate and the ratio of RNA synthesis: DNA synthesis of mammary glands associated with an increase in mammary gland DNA synthesis. On the other hand, pituitary synthesis and release of GH and prolactin, the pattern of estrous cycles and ovarian structures were affected little by these treatments. All findings strongly suggest that these tumor promoters can directly inhibit lactation by shifting mammary gland cells from a functional state to a mitotic state.

Phorbol (1–3) and its ester, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) (4,5), are known as tumor promoters: i.e., they have a promoting action on carcinogenesis, when animals are pretreated with a potent initiating stimulus (carcinogens), but show little or no tumorigenic effects, when administered alone. No data, however, are available on the effects of these agents on the physiology of normal cells, especially mammary gland cells. In this paper, we report that these tumor promoters inhibit lactation in mice by stimulating mammary gland mitosis.

Materials and methods. Highly inbred C3H/He female mice bred in our laboratory were mated with males at 2 months of age. The number of pups in each litter was adjusted to six (three females and 3 males) on the day of parturition (Day 0 of lactation). Lactating mothers were divided into three groups for phorbol and TPA (Consolidated Midland Corp., Brewster, N.Y.) and received two different doses and solvents, respectively (Table I). Each dose of phorbol (400 and 100 μg) or TPA (4 or 1 μg) was dissolved in 0.05 ml phosphate-buffered saline or dimethyl sulfoxide (DMSO) and injected subcutaneously twice daily for 2 days (Days 12 and 13 of lactation) and once on the morning of Day 14. Control animals were administered the respective solvents. Vaginal smears were checked every morning during the injection period. Throughout the experiments, animals were maintained in an animal room, which was air conditioned (24 ± 0.5° and 65–70% relative humidity) and artificially illuminated (14 hr light from 5:00 AM to 7:00 PM), and provided with a commercial diet and tap water *ad libitum*. Immediately after the last injection, the mother mouse was removed from the litter by a wire net for 4 hr, nursed again for 1 hr, and killed by decapitation.

The anterior pituitary was used for measurement of the *in vitro* incorporation of [^{14}C]leucine into growth hormone (GH) and prolactin as the indices of synthesis and release of the respective hormones by the method of Yamamoto *et al.* (6) modified by Yanai and Nagasawa (7). Each anterior pituitary was placed in a 0.15 ml of Ringer bicarbonate buffer solution (pH 7.4) containing 270 μg of glucose and 0.075 μCi of [^{14}C]leucine (311 mCi/mmole, The Radiochemical Centre, Amersham, England) and incubated for 3 hr under a constant gassing with 95% O_2–5% CO_2. After incubation, the pituitary was removed, weighed, and homogenized in 0.15 ml of distilled water. "Cold" anterior pituitary

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homogenate was added to each of the pituitary homogenate and the medium as "carrier." GH and prolactin in the pituitary and the medium were fractioned by disc electrophoresis (8). After electrophoresis, the separating layer was stained with 0.002% amido black. GH and prolactin bands were cut out and prepared for liquid scintillation counting (6). The value in the medium represents released hormone and the sum of the values in the pituitary and the medium represents the synthesized hormone. The rate of synthesis and release of each hormone were expressed in terms of cpm of incorporated [^{14}C]leucine into GH and prolactin per pituitary and per milligram of pituitary.

The *in vitro* incorporation of [^{3}H]-thymidine and [^{14}C]uridine into mammary gland DNA and RNA, respectively, was measured as the indices of the synthetic activity of DNA and RNA. At autopsy, portions of bilateral inguinal mammary tissues were removed and cut into 0.5-mm-thick slices. About 50 mg tissue was placed in 2 ml of Medium 199 containing 5 μCi [^{3}H]thymidine (5.0 Ci/mmole, The Radiochemical Centre) or 5 μCi [^{14}C]-uridine (60 mCi/mmole, The Radiochemical Centre) and incubated for 2 hr at 37° under a constant gassing with 95% O_2–5% CO_2. After incubation, nucleic acids in the tissue were extracted and 1/2 ml of extract was mixed with 10 ml of Aquasol-2 (New England Nuclear Corp., Boston, Mass.) in a scintillation vial for counting. The procedures were essentially the same as detailed previously (9).

Ovaries and uteri were weighed and ovaries were examined histologically. The statistical significance of difference between groups in each parameter was evaluated by analysis of variance and Duncan's multiple range test.

Results. While either phorbol or TPA showed no effects on the body weight of the mother, litter growth was significantly declined by the treatment with 400 μg phorbol or 4 μg TPA (Table I).

As shown in Fig. 1, daily injections of 400 μg phorbol or 4 μg TPA significantly increased the synthesis of both DNA and

TABLE I. EFFECTS OF PHORBOL AND TPA ON LITTER GROWTH RATE IN LACTATING MICE

Treatment	No. of litters	Litter growth rate (%) Days 0–12	Days 12–14
Phorbol			
400 μg	7	385 ± 21	6.8 ± 2.7[a]
100 μg	7	406 ± 14	9.0 ± 1.6
Control (PBS)	6	417 ± 12	12.5 ± 1.3[b]
TPA			
4 μg	8	384 ± 21	−1.7 ± 1.2[c]
1 μg	8	420 ± 15	5.8 ± 1.0[d]
Control (DMSO)	7	416 ± 15	8.2 ± 0.9[e]

Note. Litter growth rate was expressed in terms of the percentage change in the litter weight during the respective periods. Each value represents the means ± SE.
[a/b] $P < 0.05$.
[c/d,e/e] $P < 0.01$.

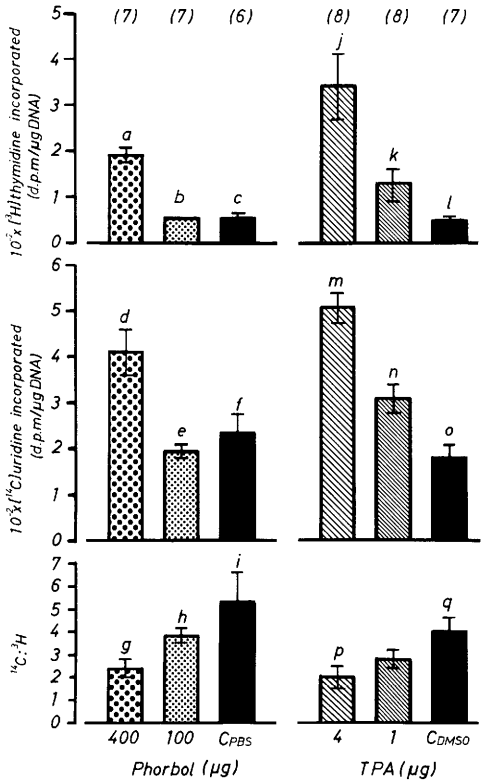

FIG. 1. Effects of phorbol and TPA on the rate of mammary gland nucleic acid synthesis in lactating mice. Each value represents the means ± SE. Number of samples is in parentheses. d/f; g/h; g/i; m/n: $P < 0.05$. j/k; n/o; p/q: $P < 0.02$. a/b; a/c; d/e; j/l; m/o: $P < 0.01$.

RNA when compared to the respective controls receiving solvents only. By contrast, the ratio of RNA synthesis: DNA synthesis (^{14}C:^{3}H) was decreased by these treatments. One microgram of TPA showed a trend similar to 4 μg TPA in both litter growth and mammary gland nucleic acid synthesis, however, the differences from the control were not statistically significant except for RNA synthesis. All parameters examined were not affected by 100 μg phorbol.

Either phorbol or TPA influenced little pituitary synthesis, release, and percentage release of GH and prolactin at doses employed.

The weights of anterior pituitary, ovaries, and uteri in mice given 400 μg phorbol or 4 μg TPA were significantly smaller than those in the respective controls except for ovarian weight in the 400 μg phorbol group (Table II). However, no difference was seen between any experimental group and the control in ovarian structures; ovaries in all groups consisted of both functional corpora lutea and several sizes of follicles. Vaginal smears were at diestrus during the injection period in all mice.

Discussion. This study shows in mice that daily subcutaneous injections of 400 μg phorbol or 4 μg TPA resulted in a significant decline in litter growth rate and the ratio of RNA synthesis: DNA synthesis associated with an increase in mammary gland DNA synthesis and no alteration of pituitary synthesis and release of GH and prolactin. The findings suggest that these tumor promoters can inhibit milk secretion by shifting mammary gland cells from a functional state to a mitotic state. While the weights of ovaries were decreased by these treatments, ovarian structures and vaginal smears were not affected. Morever, the *in vitro* stimulation by TPA of mammary gland DNA synthesis has recently been observed in immature female mice (Yanai and Nagasawa unpublished). Thus, an indirect inhibitory effects of these agents on lactation through altering ovarian hormone secretion is unlikely.

RNA synthesis promoted by 400 μg phorbol or 4 μg TPA would be ascribed to an increase in the number of cells, but not the promotion of milk synthesis, since the ratio of ^{14}C:^{3}H was significantly lower in the experimental groups than in the controls.

Whereas the cause of the significant decreases in the weights of anterior pituitary and ovaries as well as uterus is unclear at present, the direct effects of the tumor promoters on these organs to the extent of little alteration of their function is plausible.

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TABLE II. EFFECTS OF PHORBOL AND TPA ON ORGAN WEIGHTS IN LACTATING MICE

Treatment	No. of mice	Organ weights (mg/100 g body wt)		
		Anterior pituitary	Ovaries	Uterus
Phorbol				
400 μg	7	6.9 ± 0.3[a]	32.5 ± 1.3	177.9 ± 5.6[h]
100 μg	7	7.9 ± 0.4	34.7 ± 1.4	199.7 ± 6.1[i]
Control (PBS)	6	8.0 ± 0.3[b]	35.7 ± 1.7	219.9 ± 11.8[j]
TPA				
4 μg	8	6.9 ± 0.4[c]	31.9 ± 1.1[e]	181.2 ± 6.5[k]
1 μg	8	7.3 ± 0.3	36.3 ± 0.8[f]	203.4 ± 11.7
Control (DMSO)	7	7.9 ± 0.2[d]	37.9 ± 1.2[g]	226.4 ± 14.9[l]

Note. Each value represents the means ± SE.
[a,b,c,d,h,i] $P < 0.05$.
[e,f,g,h,i,j,k,l] $P < 0.01$.

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