Inhibition by Ergocornine of Initiation and Growth of 7,12-Dimethylbenzanthracene-Induced Mammary Tumors in Rats: Effect of Tumor Size (36209)

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Reports have appeared recently, while this present study was being completed, showing that administration of ergot alkaloids is capable of retarding growth of mammary tumors induced by 7, 12-diamethylbenzanthracene (DMBA) in rats (1, 2). Many tumors regressed in size under the influence of ergocornine but resumed their normal growth rate when ergocornine treatment was discontinued. In addition, many larger tumors did not appear to respond to ergocornine treatment. Ergocornine appeared to retard tumor growth by inhibiting prolactin secretion by the pituitary gland (1). These observations supported the earlier hypothesis of Meites and Nicoll (3) and Talwalker *et al.* (4) that prolactin may be the principal hormonal factor in mammary tumor development in the rat. This study was initiated to determine if ergocornine could cause complete regression of DMBA-induced mammary tumors to the extent that they would not recur, and to ascertain its influence on different sized tumors. An additional objective was to determine if ergocornine could prevent tumor induction by DMBA.

Materials and Methods. Virgin female Sprague-Dawley rats were housed in a temperature (75 ± 2°F) and light (14 hrs light, 10 hr dark) controlled room, and were given a diet of Purina Lab Chow and water *ad libitum*.

Treatment with ergocornine before mammary tumor appearance. Fifty-eight female rats, each about 45 days of age, were divided randomly into 3 groups and treated daily as follows: Group 1, 0.1 ml of corn oil; group 2, 0.4 mg of ergocornine methansulfate; group 3, 0.4 mg of ergocornine hydrogenmaleate. Both ergocornine salts were suspended in corn oil and administered subcutaneously in a 0.1 ml volume. All rats were given a single intravenous injection of 1 ml of a lipid emulsion containing 5 mg of DMBA, after 11 days of ergocornine treatment. Injections of ergocornine were continued for 5 days after DMBA treatment. All animals were palpated weekly for mammary tumors. The mean latency period for mammary tumor appearance was determined by calculating the average number of days from DMBA treatment to detection of each palpable tumor. The study was terminated 150 days after carcinogen treatment.

Treatment with ergocornine after mammary tumor appearance. Mammary tumors were induced in female rats by DMBA. Thirty-three rats with at least one palpable mammary tumor were selected, divided into 2 groups and treated daily as follows: Group 1, corn oil; Group 2, 0.3 mg of ergocornine hydrogenmaleate. Route of administration was as previously described. Each group contained many different sized tumors. At 0, 10, and 18 days after the initial treatment, rats were examined for number and size of palpable mammary tumors. Mean increase or decrease per group in number of palpable tumors was determined at 0–10, and 0–18 days after initial ergocornine treatment. Average tumor volume was calculated from measurement of the two diameters and using the formula $4/3\pi a^2 b$, which is for the volume of an oblate spheroid. This configuration closely resembled that of a tumor. Level of significance between group means was calculated by Student's t test. An attempt was made to see if the ability of ergocornine to regress tumors varied with tumor size. At the end of treatment, serum

TABLE I. Effect of Ergocornine Salts on Incidence of Mammary Tumors in Rats Treated with DMBA.

Group and treatment	No. of rats	No. of rats with tumors[a]	Total no. of tumors	Mean latency period (days)
1. Controls, corn oil	20	10	12	56.2 ± 4.0
2. Ergocornine methansulfate, 0.4 mg	19	2	2	56.0
3. Ergocornine hydrogenmaleate, 0.4 mg	19	1	1	63.0

[a] 5.0 months after DMBA treatment.

levels of prolactin were determined by radioimmunoassay. Results were expressed in terms of the NIAMD-RP-1 prolactin reference preparation.

Treatment of rats with ergocornine significantly inhibited the induction of mammary tumors in rats treated with DMBA (Table I). The mean latency period was not significantly different between groups, although the value of this statistic is doubtful because of the few tumors in the treated rats. Treatment with ergocornine also caused significant regression of mammary tumors (Table II), and over half (62%) of the mammary tumors regressed completely. Palpation of ergocornine-treated rats 2 months after termination of treatment revealed that 64% of the tumors that regressed completely (to palpation) did not recur. Tumors which were present at the end of the treatment period, but demonstrated some growth inhibition, continued to grow after the treatment was dicontinued. In general, tumors about 1.8 cm^3 in volume and smaller regressed rapidly and disappeared in response to ergocornine treatment; whereas tumors of about 14.1 cm^3 and larger were relatively uninhibited. Severe growth inhibition and variable degrees of regression were noted in tumors with areas intermediate between these two values. This dose of ergocornine, when administered as an oil suspension, had no adverse effects on these animals. Although the treated animals did not appear to gain weight as well as the controls, there was no statistical difference in body weights at the end of the treatment period.

Discussion. When ergocornine injections were begun before DMBA treatment, a highly significant decrease in incidence of mammary tumors was observed. The decreased tumor incidence was due to inhibition of prolactin secretion from the anterior pituitary. The process of mammary tumorigenesis appears to be dependent on the presence of prolactin, since a reduction in prolactin secretion by a number of other means, such as ovariectomy (5, 6) or hypophysectomy (7, 8), was shown to inhibit mammary tumor formation and cause regression of established mammary tumors. Treatments which stimulate prolactin secretion, and that are initiated after DMBA treatment, have been shown to cause dramatic increases in mammary tumor growth (9, 10). Median eminence lesions in rats previously treated with DMBA causes rapid growth of already established tumors and hastens the appearance of new tumors (11).

Ergocornine administration after tumor appearance caused a consistent and rapid regression of tumors up to about 1.8 cm^3. Most of these tumors disappeared to palpation. Over half of the tumors did not recur as indicated by palpation 2 months after treatment. Since 36% of the tumors did recur, it appears that 18 days' treatment was not sufficient to cause regression of all of the tumor cells. Interestingly, a significant incidence of complete tumor regression was not obtained in the other studies with ergot alkaloids (1, 2), while the present study demonstrates a much more rapid and complete regression. This difference may be due to the mode of administration of the compound. In this study, ergocornine was suspended in corn oil; however, in the other studies it was dissolved in saline plus alcohol

or in 20% dimethylsulfoxide. Since the water solubility of this drug is very low, it was absorbed from the oil suspension at a very slow rate and was therefore capable of inhibiting prolactin secretion during an entire 24 hr period. In contrast, when ergocornine was given in solution the entire quantity of the drug was immediately available to the animal metabolism and may have been inactivated or excreted after a few hours. Thus, prolonged absorption of the drug may be necessary to keep prolactin levels low and cause rapid regression of prolactin dependent tumors. In support of this are our findings that prolactin levels are low 24 hr after injection of a corn oil suspension of ergocornine.

When tumors reached about 14.1 cm^3 in volume, administration of ergocornine did not cause tumor regression. However, the growth rates were somewhat lower than for untreated tumors. Apparently these tumors were no longer hormonally dependent.

At this point in time extrapolation from rodents to humans or other species is difficult. There are strong indications, however, that the early stages of breast cancer in humans may be hormonally dependent. Hypophysectomy, ovariectomy, and adrenalectomy are treatments used to inhibit the growth of breast cancers. Many of these procedures, at least hypophysectomy, are not resorted to until the disease is in its advanced stages. At this stage, much of the hormonal dependence is lost. If therapy with a compound that inhibits prolactin, such as ergocornine, were begun in the very early stages of the cancer, while it is still dependent on prolactin, significant or possibly complete regression might result.

Summary. Administration of ergocornine 11 days before, and 5 days after, treatment with DMBA significantly inhibited the induction of mammary tumors in rats. Ergocornine caused rapid and complete (to palpation) regression of 62% of established, DMBA-induced mammary tumors. Examination of sites where tumors had completely regressed to palpation, 2 months after ergocornine treatment was discontinued, revealed that 64% of the tumors did not recur. The effectiveness of ergocornine in regressing tumors was found to be related to initial tu-

TABLE II. Effects of Ergocornine on Growth and Development of DMBA-Induced Mammary Tumors in Female Rats.

Group and treatment	Total no. of rats	Av no. of tumors/rat			No. of rats with tumors after 18 days treatment	Av tumor vol/rat after 18 days (cm^3)	Serum prolactin levels (ng/ml)
		Initial	10 days treatment	18 days treatment			
1. Controls corn oil, 0.1 ml	15	1.4 ± 0.2[a]	2.2 ± 0.2	2.7 ± 0.4	15	4.5 ± 2.3	80.2 ± 9.4
2. Ergocornine hydrogenmateate, 0.3 mg	18	1.6 ± 0.2	0.7 ± 0.1[b]	0.6 ± 0.2[b]	8	0.20 ± 0.1[c]	9.5 ± 1.3[b]

[a] Mean ± SE.
[b] Significantly different from control value: $p < .001$; [c] $p < .05$.

mor size. Tumors with volumes up to 1.8 cm^3 regressed rapidly after ergocornine; whereas tumors approximately 14.1 cm^3 and over did not respond to treatment. Tumors intermediate between these two sizes showed growth inhibition, and many showed regression in varying degrees. Therefore, mammary tumors start to lose their hormonal dependence as they become larger, and eventually may lose it completely. In view of these results, we suggest that prolactin inhibitors may cause regression of breast cancer in its early stages and may prevent formation of tumors through prophylactic treatment.

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1. Nagasawa, H., and Meites, J., Proc. Soc. Exp. Biol. Med. **135**, 469 (1970).

2. Heuson, J. C., Wallbroeck-Van Gaver, C., and Legros, N., Eur. J. Cancer **6**, 353 (1970).

3. Meites, J., and Nicoll, C. S., Annu. Rev. Physiol. **28**, 57 (1966).

4. Talwalker, P. K., Meites, J., and Mizuno, H., Proc. Soc. Exp. Biol. Med. **116**, 531 (1964).

5. Huggins, C., Briziarelli, G., and Sutton, H., J. Exp. Med. **109**, 25 (1959).

6. Dao, T. L., Cancer Res. **22**, 973 (1962).

7. Furth, J., and Clifton, K. H., Cancer **10**, 842 (1957).

8. Sterental, A., Dominguez, V. M., Weismann, C., and Pearson, O. H., Cancer Res. **23**, 481 (1963).

9. Welsch, C. W., Clemens, J. A., and Meites, J., Cancer Res. **29**, 1541 (1969).

10. Welsch, C. W., and Meites, J., Experentia **26**, 1133 (1970).

11. Clemens, J. A., Welsch, C. W., and Meites, J., Proc. Soc. Exp. Biol. Med. **127**, 969 (1968).

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