Mammary Tumor Induction by Estrogen or Anterior Pituitary Hormones in Ovariectomized Rats Given 7, 12-Dimethyl-1, 2-Benzanthracene.* (29297)

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Ovarian and pituitary hormones play a major role in the normal development of the mammary gland, and in maintenance and growth of established mammary tumors in the rat(1-8). The pituitary appears to be of primary importance in mammary growth in the rat, since a combination of growth hormone (STH) and prolactin(9) or transplantation of a pituitary "mammotropic" tumor (10; also Talwalker, Meites and Deuben, unpublished), which presumably secretes prolactin and STH(5,11,12), induced mammary growth in adreno-gonadectomized rats. On the other hand, ovarian or adrenal steroids have little or no effect on mammary growth in absence of the pituitary(1-3). Induction of mammary growth by estrogen in intact rats appears to be partially mediated through stimulation of pituitary prolactin and STH secretion(3,4).

Rapid induction of mammary carcinomas by intragastric administration of 3-methylcholanthrene (3-MC) or 7,12-dimethyl-1,2-benzanthracene (DMBA) has been described (13,14). Hypophysectomy prevents mammary tumor induction by 3-MC, whereas tumors can be induced in hypophysectomized rats provided estrogen, progesterone and STH are given together with 3-MC(15). Dao(16) reported that ovariectomy before a single feeding of DMBA prevented mammary tumor induction in the Sprague-Dawley female rat, but tumors appeared when ovaries were transplanted concurrently or within several days after DMBA administration. He concluded that ovarian hormones are essential for mammary tumor induction by DMBA. Since ovarian hormones appear to influence pituitary prolactin and STH secretion, it was of interest to determine the role of estrogen, or prolactin and STH, in mammary tumors induced by a single feeding of DMBA in ovariectomized Sprague-Dawley rats.

Methods. Sprague-Dawley, virgin female rats (Hormone Assay Laboratories, Chicago, Ill.) were randomly divided into 6 groups. When they reached 52 days of age, Group 1 was sham-operated, while Groups 2-6 were bilaterally ovariectomized. Seven days later, all rats (Groups 1-6) received, once only, 20 mg of 7, 12-dimethyl-1,2-benzanthracene (DMBA, Eastman Organic Chemicals, Rochester, N. Y.) in 1 ml corn oil, by intragastric instillation. This dose of DMBA is considered optimal, since 100% of rats so treated develop mammary tumors(14). Groups 1-5 were treated for 7 days before and 7 days after (total 14 days) DMBA administration, as shown in Table I. Group 6 was treated, daily, with porcine STH‖ and ovine prolactin‖ in increasing dosages, beginning a day after ovariectomy and continuing as follows: Days 1-15, 1 mg each; days 16-30, 1.5 mg each; days 31-45, 2 mg each; days 46-60, 2.5 mg each; and days 61-75, 3 mg each. All injections were given subcutaneously. Estradiol was injected in 0.1 ml corn oil; and porcine STH and ovine prolactin (15 IU/mg) were given in 0.2 ml saline.

The rats were examined for tumors by palpation twice weekly. Occurrence of the tumor was dated from the day of feeding DMBA. The number and percent of animals developing tumors (incidence) was determined at the end of 40 weeks, and the range

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TABLE I. Effects of Hormone Administration on Mammary Tumor Induction in Ovariectomized Rats Treated with 7,12-Dimethyl-1,2-Benzanthracene (DMBA).

Group and treatment*	Duration of treatment (days)	Total No. of rats	No. and % of rats with tumors	Avg No. of tumors per tumor-bearing rat	Range and mean latency period (days)
1. Sham-operated controls, saline	14	18	18(100)	3.9	52–110 (68)
2. Ovax-controls, saline	14	20	0 (0)	—	—
3. Ovax, 1 μg Esd 1 × daily	14	12	4 (33)	1.5	72–130 (104)
4. " , 10 μg Esd 1 × daily	14	13	3 (23)	1.0	76–102 (89)
5. " , 1 mg STH + 1 mg prolactin 2 × daily	14	9	4 (44)	1.8	114–210 (146)
6. " , STH + prolactin† 1 × daily	75	9	6 (66)	2.3	94–207 (122)

* Ovax = ovariectomized; STH = porcine growth hormone; Esd = estradiol. DMBA was administered 7 days after ovariectomy.
† Dosages of STH and prolactin were increased every 15 days. See text for details.

and mean time of appearance of first tumors (latency) were calculated. Rats were killed when they appeared ill, or when the tumors became so large that they ulcerated and bled. At necropsy, the tumors were fixed in 10% neutral formalin, sectioned at 6 μ, and stained with hematoxylin and eosin.

Results. The results are summarized in Table I. When 20 mg of DMBA were fed to the intact sham-operated controls and they were thereafter given saline (Group 1), 100% of the animals developed mammary tumors within 52-110 (mean 68) days. Ovariectomy 7 days before DMBA administration completely prevented tumor induction (Group 2). Limited treatment of ovariectomized rats with 1 or 10 μg estradiol (Groups 3 and 4) increased mammary tumor incidence to 33 and 23%, respectively. In the estrogen treated groups, the mean latency period was slightly higher and average number of tumors per tumor-bearing rat was lower than in the sham-operated intact controls. Combined daily treatment with STH and prolactin, either for 14 or 75 days (Groups 5 and 6), increased mammary tumor incidence to 44 and 66%, respectively. In these rats also, the mean latency period was higher and the average number of tumors per tumor-bearing rat was lower than in the sham-operated controls.

Spontaneous mammary carcinomas in Sprague-Dawley rats occur very rarely(13, 14,17). Mammary carcinomas were the only type observed in these DMBA-fed rats. No fibrosarcomas were detected in the mammary region. The mammary carcinomas were grossly nodular, white and soft. Hemorrhage and central necrosis were often encountered, especially when the tumors became large. The tumors contained acini lined with many layers of epithelial cells arranged to form gland-like structures with papillary projections in the lumina. The lumina were often filled with eosinophilic-staining material. Metastasis of these mammary tumors was not observed, but often infiltration of adjacent muscles and skin was found. In all respects these tumors were similar to those previously described in Sprague-Dawley rats treated with MC or DMBA(13,14). The principal causes of death of the rats appeared to be due to hemorrhage and necrosis of large tumors, and increased susceptibility to respiratory infection.

Discussion. Recent investigations have led to the concept that mammary carcinogenesis involves two separate phases (a) initiation of carcinogenesis by a carcinogen and (b) promotion of tumor growth by hormones. Carcinogens are believed to bring about an irreversible change of the mammary cells, whereas hormones presumably regulate growth and function of these cells. Hormones themselves apparently do not induce mammary carcinogenesis(4,16). The results of the present study show that estradiol, or prolactin and STH, administered during the initiating phase of mammary carcinogenesis by DMBA, permitted ovariectomized rats to

develop mammary carcinomas. Thus neoplastic transformation of the mammary gland of these rats required the participation of hormones, particularly prolactin and STH. Estrogen may participate in the initiating phase through stimulation of prolactin and STH secretion by the pituitary, and also by a direct action on the mammary gland.

It is evident from the present study that estrogen, or prolactin and STH, did not induce mammary tumors in all of the ovariectomized rats fed DMBA. This may be due to the particular doses of hormones used, which may not have been optimal, and to the schedule and duration of hormone treatments. It is also possible that other ovarian or pituitary hormones may favor mammary carcinogenesis in the rat. However, our study clearly shows that limited treatment with the pituitary hormones, prolactin and STH, in the absence of the ovaries, can induce mammary tumors in rats given a single feeding of DMBA. The possibility that adrenal cortical hormones participated in induction of mammary tumors in these rats cannot be absolutely excluded, since the adrenals were not removed. However, the complete absence of mammary tumors in the ovariectomized rats given DMBA, and the relative purity of the STH and prolactin preparations used, would seem to exclude this possibility. Adrenalectomy of Sprague-Dawley rats prior to DMBA feeding was found to result in death of the animals.

Mammary tumors induced by 3-MC or DMBA feeding in rats are hormone dependent during the early stages of growth, hence they may regress when appropriate endocrine glands supplying the hormones are removed. Thus, growth of mammary tumors is inhibited by overiectomy, ovariectomy and adrenalectomy or by hypophysectomy, and stimulated by prolactin and STH secreting pituitary "mammotropic" tumor transplants(4-8, 13,14). Estrogen maintains or stimulates growth of these mammary tumors only in the presence of the pituitary(18). Therefore, estrogen may promote growth of these mammary tumors through stimulation of prolactin and STH secretion by the pituitary. The present study, together with others(4-6,15, 18), suggests that the pituitary hormones, prolactin and STH, are of primary importance both in the initiating and promoting phases of mammary carcinogenesis in rats fed 3-MC or DMBA.

Summary. The effects of estrogen, or prolactin and STH, on mammary tumor induction by a single feeding of 20 mg of 7, 12-dimethyl-1,2-benzanthracene (DMBA) in ovariectomized Sprague-Dawley rat was investigated. After DMBA administration, mammary tumor incidence in sham-operated controls was 100%. In ovariectomized rats, DMBA failed to induce any mammary tumors by the end of 40 weeks. However, daily treatment with 1 or 10 μg estradiol, for 7 days before and after (total 14 days) DMBA administration, induced mammary tumors in 33 and 23% of rats, respectively. Similar treatment with 1 mg STH and 1 mg prolactin, twice daily for 14 days, elicited mammary tumors in 44% of the rats, while continuous daily treatment with increasing amounts of prolactin and STH for 75 days, resulted in a 66% incidence of mammary tumors. The average number of tumors per tumor-bearing rat was lower and mean tumor latency was longer in the ovariectomized rats given estrogen or STH and prolactin than in sham-operated controls. All tumors were mammary carcinomas. These results indicate that the 2 pituitary hormones, STH and prolactin, can induce mammary carcinogenesis in DMBA-treated rats in the absence of the ovaries. They also suggest that these 2 hormones, as well as estrogen, participate in the initiating and promoting phases of mammary carcinogenesis; and that the effects of estrogen may be partially mediated through stimulation of STH and prolactin secretion by the pituitary.

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Taurine Excretion Following Drug-Induced Muscle Necrosis and X-Irradiation.* (29298)

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Excretion of large amounts of taurine in the urine is a frequent, if not constant, sequel to necrosis of striated muscle(1,2). Skeletal muscle contains more than 75% of the total body taurine(3), but its precise role in the metabolism of the muscle cell is unknown.

In a previous paper(1), the taurinuria of muscle injury was attributed to an abnormal breakdown of taurine precursors. The occurrence of abnormal taurinuria following irradiation(3-6), and after administration of colchicine(7), suggests however that a generalized metabolic disturbance, rather than a specific disorder of muscle, may be implicated.

The purpose of this study was comparison of taurine excretion in the rat after administration of plasmocid and isoproterenol and after whole body irradiation. The quantity of taurine in certain muscles of normal rats and of rats given plasmocid was also compared.

Plasmocid (8-3-diethylaminopropylamino-6-methoxyquinoline) dihydroiodide induces a constant pattern of muscle necrosis in the rat. The diaphragm and heart are much more severely damaged than the limb muscles(2,8,9). The lesions of isoproterenol (1-(3,4-dihydroxyphenol-2-isopropylaminoethanol) dihydrochloride) are limited to the myocardium (10,11).

Materials and methods. White female rats of the Saint Louis University strain, weighing 150-250 g, were used. The animals were fasted during the 24-hour period of the experiment; water was not limited. Twelve rats each received a single intraperitoneal injection of plasmocid, 25 mg/kg: 6 were injected subcutaneously with isoproterenol, 85 mg/kg: 6 were radiated with 800 r: 18 animals served as controls.

The animals were kept in individual metabolic cages in which the urine, uncontaminated with feces, was collected in test tubes containing crystals of thymol. Collection was started at 9 A.M. immediately after injection of the drug or the radiation. The tubes were changed every 4 hours and the samples stored at 4°C until analyzed. Twenty-four hours later the animals were killed by exsanguination, the muscles quickly excised and aqueous extracts of heart, diaphragm, and quadriceps prepared by the method of Awapara(12). Portions of these muscles were also fixed in Zenker's solution for microscopic examination.

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