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*Letter to the Editor*

# Effect of Submandibular Gland Removal on Carcinogen Induced Rat Mammary Tumors

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RECENTLY it has been reported that the removal of the submandibular glands of mice can inhibit the appearance of spontaneous mammary tumors and slow the growth of pre-existing breast tumors [1]. The mechanism underlying this effect was not clearly demonstrated in that study, but it was hypothesized that it was due to a decrease in growth factors known to be produced by the submandibular gland. There is known to be an epidemiologic association between salivary gland tumors in humans and breast cancer [2, 3]. The implications of these observations are that manipulations of the products of salivary glands or possibly their removal might provide therapeutic strategies in the treatment of human breast cancer. We investigated the possibility of using dimethylbenz(a)anthracene (DMBA) induced mammary tumors in the rat as a model system to study the role of the salivary glands in mammary tumor growth.

Mammary tumors were initiated in 100 50-day old female Sprague-Dawley rats by giving them 20 mg of DMBA in 2 ml of peanut oil by gavage. The surviving 97 animals were split into three groups. Bilateral submandibular gland removal was performed on 20 animals at 64 days of age. Twenty animals were sham operated. The remaining animals had no anesthesia (diethyl ether) or surgery. Rats were weighed and examined for tumors approximately every 7 days starting 4 weeks after treatment with the DMBA. Animals were sacrificed 12 weeks after DMBA treatment and blood was collected for hormone studies. Estradiol determinations were done using a radioimmunoassay kit produced by Diagnostics Products Corporation (Los Angeles, California, U.S.A.).

We did not find any evidence that submandibumandibular gland removal inhibited the growth of tumors in the rat that had been initiated by DMBA. Figure 1 shows that both the sham operated and the group with the submandibular glands removed had a higher tumor incidence than the non-operated control group. Thus no clear effect of submandibular gland removal could be demonstrated. Twelve weeks after DMBA administration the average tumor sizes were 100 $mm^2$ ($n = 22$) in the submandibular gland removed rats, 102 $mm^2$ ($n = 20$) in the sham operated group, and 64 $mm^2$ ($n = 38$) in the non-operated controls. Thus submandibular gland removal did not cause a decrease in tumor size either.

Bhattacharyya *et al.* [4] reported that submandibular gland removal resulted in elevations

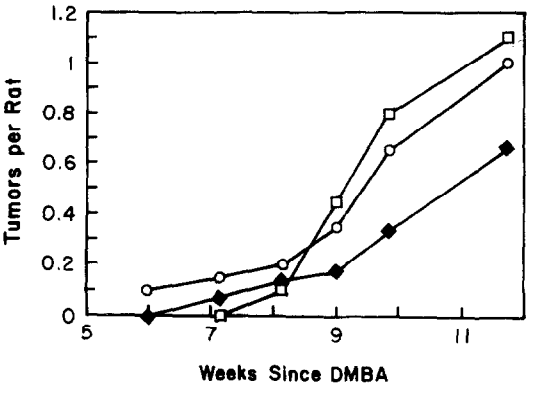


*Fig. 1. Tumor incidence in the DMBA treated rats after submandibular gland removal (□), sham operation (○), and no surgical manipulation (◆).*

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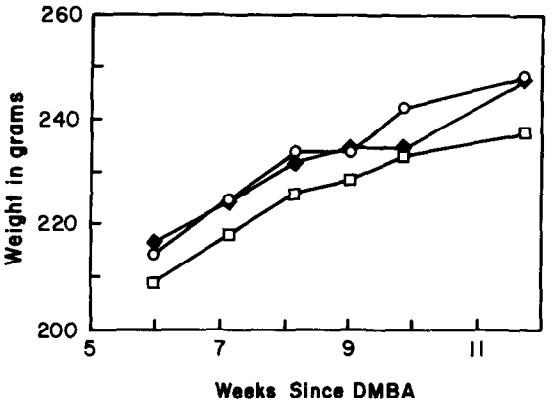


*Fig. 2. Weights of DMBA treated rats after submandibular gland removal* (□), *sham operation* (○), *and no surgical manipulation* (◆).

in adolescent rat serum estradiol levels so we measured these levels in the operated and sham operated groups at 5 months of age (fully adult). The levels were 22 + 8 ($n$ = 16) and 27 + 10 pg/ml ($n$ = 15) respectively. Similarly we did not observe any striking differences in the weights of the different experimental groups of rats (Fig. 2).

We did not find that submandibular gland removal caused a decreased incidence or smaller size of mammary tumors in DMBA treated rats. Thus, at least in this model system, the presence or absence of submandibular glands does not influence breast tumor incidence or growth. This is in contrast to the results of Kurachi *et al.* [1] who investigated the results of submandibular gland removal on spontaneously arising tumors in mice. The reason for this difference is unclear. Perhaps the estradiol and prolactin dependent tumors induced by DMBA in the rat can grow independently of peptide hormones produced by the rat submandibular gland in the otherwise endocrinologically intact animal. Further we have demonstrated that the submandibular gland does not strongly affect estradiol levels in the rat and that it does not seem to produce a cofactor that allows the tumor to be driven by estrogen.

It should be noted that the exact endocrinologic role of the submandibular gland is unknown. For example, although epidermal growth factor (EGF) is found in high concentrations in this gland, it is also found in high concentrations in other tissues such as Brunner's glands of the duodenum [5]. Removal of submandibular glands of mice caused only a 25% fall in serum EGF levels [6]. There is conflicting evidence of the importance of the salivary glands in maintaining EGF levels in breast milk with some reports showing no change in EGF levels of sialoadenectomized mice [7, 8] and a report showing a 50% decrease after this procedure [9].

We did note in the course of this study that both groups of operated rats had a higher tumor incidence than the non-operated control group. This stimulation of breast tumor growth after surgery has been noted before [10] and may be due to stress induced increases in prolactin or other phenomena.

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