PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 368–377 (1987)

Influence of Submandibular Salivary Glands on Hormone Responsiveness of Mouse Mammary Glands[1] (42627)

LEWIS G. SHEFFIELD AND CLIFFORD W. WELSCH

Dairy Science Department, University of Wisconsin, Madison, Wisconsin 53706, and Department of Anatomy, Michigan State University, East Lansing, Michigan 48824

Abstract. Surgical removal of the submandibular salivary glands (sialoadenectomy) of female Balb/c mice significantly ($P < 0.05$) reduced mammary development as judged by development scores and mammae DNA levels. Reduction in mammae development score by sialoadenectomy was observed in both mice saline injected and mice treated with estradiol and progesterone. Autografts of submandibular salivary tissue or daily administration of EGF to sialoadenectomized mice partly alleviated the atrophy of the mammary gland induced by sialoadenectomy ($P < 0.05$). The results of our studies are consistent with a model of mammary gland developmental regulation that includes the submandibular salivary gland as a mediator of mammogenesis via secretion of EGF. © 1987 Society for Experimental Biology and Medicine.

Epidermal growth factor (EGF) is a single-chain, 6045-Da polypeptide found in high concentrations in the submandibular salivary glands of mice (1). The concentration of EGF in both serum and submandibular salivary glands increases during pregnancy in both humans and mice (2, 3). Receptors for EGF have been identified in mouse mammary tissue and shown to increase during early pregnancy (4). Recent results also indicate that mammary EGF receptors are regulated by thyroid hormones (5). EGF has been shown to increase growth and inhibit differentiation of mouse mammary tissue *in vitro* (6). These results strongly suggest a role for EGF in regulating mammary gland development.

Okamoto and Oka (7) observed that removal of submandibular salivary glands (sialoadenectomy) of female mice reduced subsequent survival of offspring. Results of Okamoto and Oka (7) indicated that the effect of sialoadenectomy was due to reduced milk production, which was caused by reduced mammary gland size. This effect of sialoadenectomy could be alleviated by administration of EGF, indicating that EGF is important for normal *in vivo* mammary development.

The study by Okamoto and Oka (7) examined offspring survival and mammary size during lactation. The objective of the study reported in this communication is to determine the effect of sialoadenectomy on the ability of the mammary gland to respond to a refined mammogenic hormonal stimulus *in vivo* (i.e., estrogen + progesterone treatment).

Materials and Methods. *Trial 1.* Female Balb/c mice (5 weeks of age) were obtained from Charles Rivers Laboratories (Wilmington, MA). Mice were sham operated, sialoadenectomized (submandibular salivary glands removed, other salivary glands left in place), or sialoadenectomized, and submandibular salivary tissue was cut into 3- to 5-mm^3 pieces and autografted subcutaneously (salivary transplants). In the group receiving salivary transplants, all of the submandibular salivary tissue from a given mouse was autografted to that mouse. After 2 weeks for surgical recovery, mice were injected subcutaneously daily for 14 days with saline (0.9% NaCl solution), (control) or 17β-estradiol (1 μg/day) + progesterone (1 mg/day, E + P, Sigma Chemical Co., St. Louis, MO) (10 per treatment).

The day after the last injection mice were killed by decapitation. Trunk blood was collected and serum analyzed for EGF by radioimmunoassay using a modification of the procedure employed by Kurachi and Oka (3). Because of possible circadian rythms in

---

[1] Supported by Grant HD-17331 from the National Institutes of Health and Grant IN-35-28-9 from the American Cancer Society.

0037-9727/87 $1.50

Copyright © 1987 by the Society for Experimental Biology and Medicine.
All rights reserved.

serum EGF concentration (3), mice were maintained on a 12-hr light:12-hr dark photoperiod. Mice were killed within about 1 hr of each other, beginning about 1 hr before the beginning of the light phase of the photoperiod (i.e., while serum EGF concentrations were high). Assay buffer used for EGF determination was 0.05 *M* phosphate-buffered (pH 7.4) saline (0.9% NaCl) containing 1% bovine serum albumin (radioimmunoassay grade, Sigma) and 0.1% sodium azide. Each assay tube contained 50 μl of standard EGF (receptor grade, Sigma) or mouse serum (undiluted or diluted 1:4 with assay buffer), 100 μl ^{125}I-EGF (25,000 cpm/tube diluted in assay buffer, (Amersham Co., Arlington Heights, IL) and 100 μl rabbit anti-EGF (diluted 1:100,000 in assay buffer containing 4% normal rabbit serum). Each sample was assayed in duplicate. The assay tubes were incubated 48 hr at 5°C. Sheep anti-rabbit serum (diluted 1:7 in assay buffer), (Sigma) was added to each tube (100 μl) and the assay tubes were incubated an additional 24 hr. Tubes were then centrifuged (4000g for 15 min), the pellet was washed in cold assay buffer, and the tubes (representing bound EGF) were counted in a gamma ray spectrometer. This procedure resulted in an assay detection limit of approximately 10 pg/tube (0.2 ng/ml undiluted serum). Within the limits of the assay, 1:4 dilution of samples did not affect results. Intra- and interassay coefficients of variation were 10 and 13%, respectively. Addition of known quantities of purified (receptor grade) EGF to pooled mouse serum gave the expected increase in EGF (within the limits of the assay).

One number 4 (inguinal) mammary gland was removed, fixed, stained with carmine–alum (Sigma), and the development score was determined. Mammary development was rated as described previously (8) on a scale of 1 to 6 using the following criteria: 1 = few ducts, few or no end buds; 2 = moderate duct growth, moderate number of end buds; 3 = numerous ducts and branches, many end buds; 4 = numerous ducts and branches, minimum lobule–alveolar development; 5 = numerous ducts and branches, moderate lobule–alveolar growth; and 6 = numerous ducts and branches, dense lobule–alveolar development as in late pregnancy.

Lymph nodes were carefully removed from the contralateral fourth and fifth mammary glands. The glands were homogenized in 0.9% (w/v) NaCl and DNA content was determined by the diphenylamine reaction (9).

Trial 2. Female Balb/c mice were obtained at 21 days of age and fed a liquid diet (Bioserve, Inc., French Town, NJ) *ad libitum* for the duration of the experiment. At 5 weeks of age, mice were ovariectomized and either sham operated, sialoadenectomized, or sialoadenectomized, and salivary tissue was autografted subcutaneously as described for the first trial (six mice per group). After 2 weeks recovery, all mice were injected with E + P for 14 days. Mice were killed and serum EGF, mammary gland development score, and mammary DNA were determined as previously described.

Trial 3. Female Balb/c mice were fed liquid diets from weaning, as in Trial 2. Mice were ovariectomized and either sham operated or sialoadenectomized at 5 weeks of age. After 2 weeks for surgical recovery, all mice were injected daily for 14 days with E + P. In addition, half of the sialoadenectomized mice were injected daily subcutaneously with mouse EGF (5 μg/day, Sigma, six mice per group). Mice were then killed and serum EGF, mammary gland development score, and mammary DNA were determined as previously described.

Statistical analysis. Data from the three trials were analyzed separately by analysis of variance. Planned comparisons were used to compare sialoadenectomy with sham operation and salivary transplant or EGF treatment with both sham operation and sialoadenectomy. Data are presented as means ± standard error. Unless otherwise noted, significance was set at $P < 0.05$. In all cases, two-sided tests were performed testing the alternative hypothesis that treatment means were not equal against the null hypothesis that treatment means were equal (10).

Results. *Trial 1.* Body weights of sham-operated mice were 18.8 ± 0.7 for saline-treated mice and 22.0 ± 0.9 for E + P-treated mice. Sialoadenectomy resulted in body weights of 17.8 ± 1.1 (saline) and 19.2 ± 0.5 (E + P), while sialoadenectomy and salivary transplant resulted in body weights of 17.2 ± 0.5 (saline) and 19.6 ± 0.6 (E + P). Thus, body weights of sialoadenectomized mice averaged 7% less than body weights of sham-operated mice. This difference was not significant at $P < 0.05$ but was significant at $P < 0.10$. Salivary transplantation did not restore body weights. In addition, sialoadenectomy reduced serum EGF concentrations to undetectable concentrations in 7 of 10 saline-treated and 6 of 10 E + P-treated mice (Fig. 1). In sialoadenectomized mice with detectable serum EGF, the EGF concentration was low relative to controls (2 mice with ≈ 1ng/ml, remaining 5 mice with <0.5 ng/ml EGF). Our EGF assay was capable of detecting 0.2 ng/ml EGF. Serum EGF was detectable in all mice with salivary transplants, indicating that the transplanted salivary tissue retained the ability to produce EGF, although levels were low relative to sham-operated mice. In addition, autografted salivary tissue retained normal histological morphology. However, while E + P treatment appeared to increase serum EGF concentrations in sham-operated mice ($P < 0.05$), no such increase was statistically detectable in sialoadenectomized mice with salivary gland transplants.

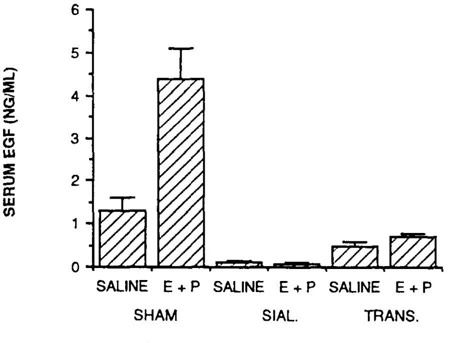

FIG. 1. Serum epidermal growth factor concentration of mice sham operated (SHAM), sialoadenectomized (SIAL.), or sialoadenectomized and given subcutaneous salivary gland transplants (TRANS.). The mice were injected daily for 14 days with saline or estradiol + progesterone (E + P). Results are presented as means ± standard error. E + P increased serum EGF in sham-operated mice ($P < 0.05$). Sialoadenectomy reduced serum EGF ($P < 0.05$). Trial 1; $n = 10$ mice/group.

Whole mount examination of mammary glands showed that sialoadenectomy reduced gland development (Fig. 2). In sialodenectomized–saline-treated mice, mammary ducts were thin and atrophic (compared to sham-operated mice). Salivary transplant resulted in mammary ducts that were thicker and more highly developed than that observed in sialoadenectomized mice. E + P injections increased mammary development in sham-operated, sialoadenectomized, and salivary transplant groups. However, mammae of sialoadenectomized mice were less developed than glands from the other two groups. In particular, ducts did not completely fill the fat pad in sialoadenectomized mice, while they did in sham-operated mice and mice with salivary transplants.

Mammary whole mount development scores (Fig. 3) reflected the effects of sialoadenectomy discussed above. Sialoadenectomy reduced development score from 2.2 ± 0.1 (sham operated, saline treated) to 1.1 ± 0.1 (sialoadenectomized, saline treated) ($P < 0.05$). Salivary transplant restored development score to values not significantly different from those observed in sham-operated mice (Fig. 3). Among all surgical groups (i.e., sham operation, sialoadenectomy, salivary transplant), E + P significantly increased mammary development. However, among E + P-treated mice, mammary development score was reduced by sialoadenectomy (from 4.7 ± 0.1 in sham-operated mice to 3.7 ± 0.2 in sialoadenectomized mice, ($P < 0.05$). Salivary transplants increased development score to control values.

In sham-operated mice, E + P increased mammary DNA by 96 μg/gland ($P < 0.05$). In sialoadenectomized mice, mammary DNA was increased by 51 μg/gland (53% as large an increase as observed in sham-operated mice). This increase was significantly greater than zero ($P < 0.05$) but significantly less than the increase observed in sham-operated mice ($P < 0.05$). In mice with salivary transplants, mammary DNA was increased by 89 μg/gland, which was not significantly different from the increase observed in sham-operated mice but was different from the increase observed in sialoadenectomized mice at $P = 0.06$. These differences in

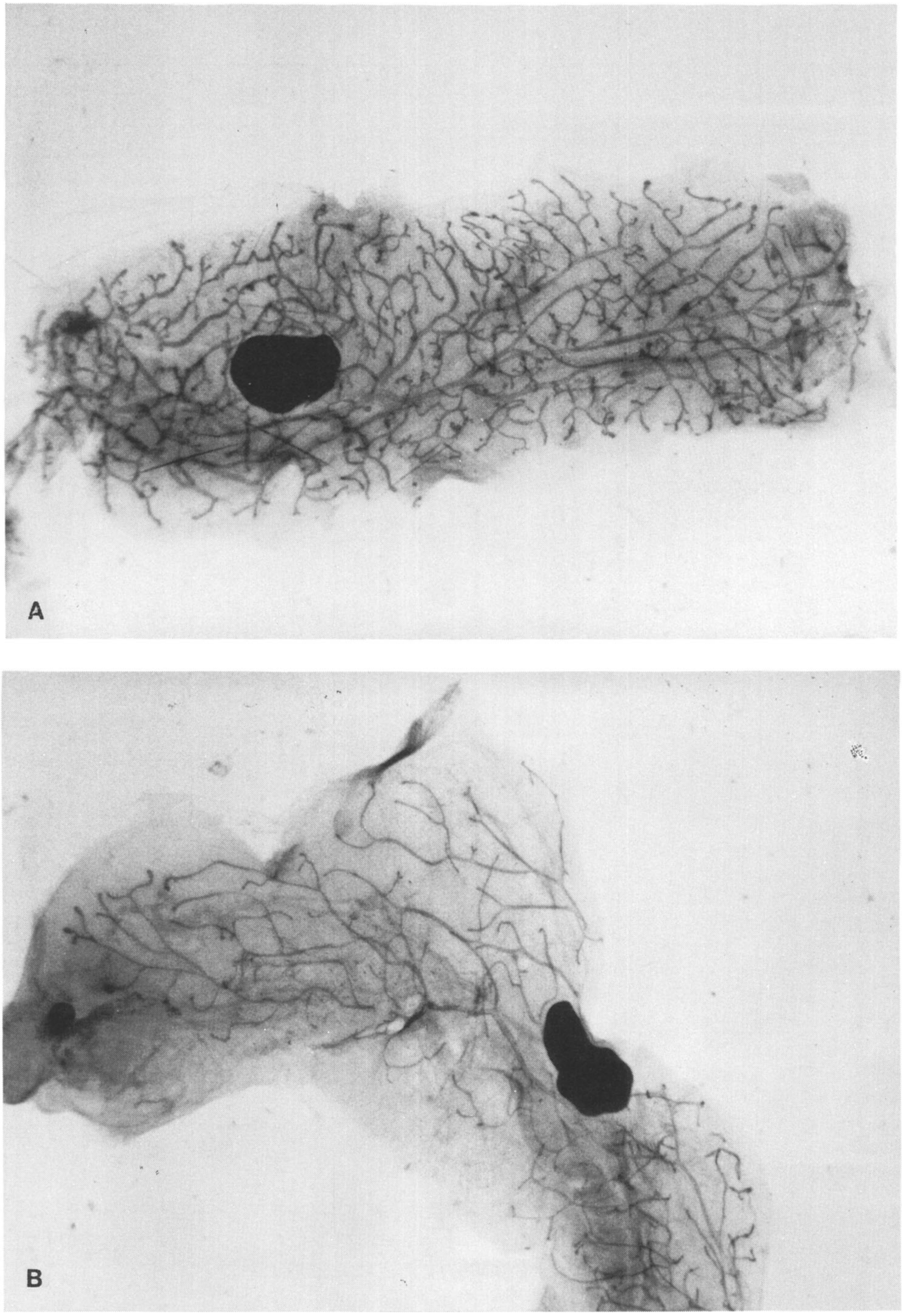

FIG. 2. Representative whole mounts of mouse mammary glands. A, sham operated, saline treated; B, sialoadenectomized, saline treated; C, salivary transplant, saline treated; D, sham operated, estrogen + progesterone treated; E, sialoadenectomized, estrogen + progesterone treated; F, salivary transplant, estrogen + progesterone treated. (×16). Trial 1.

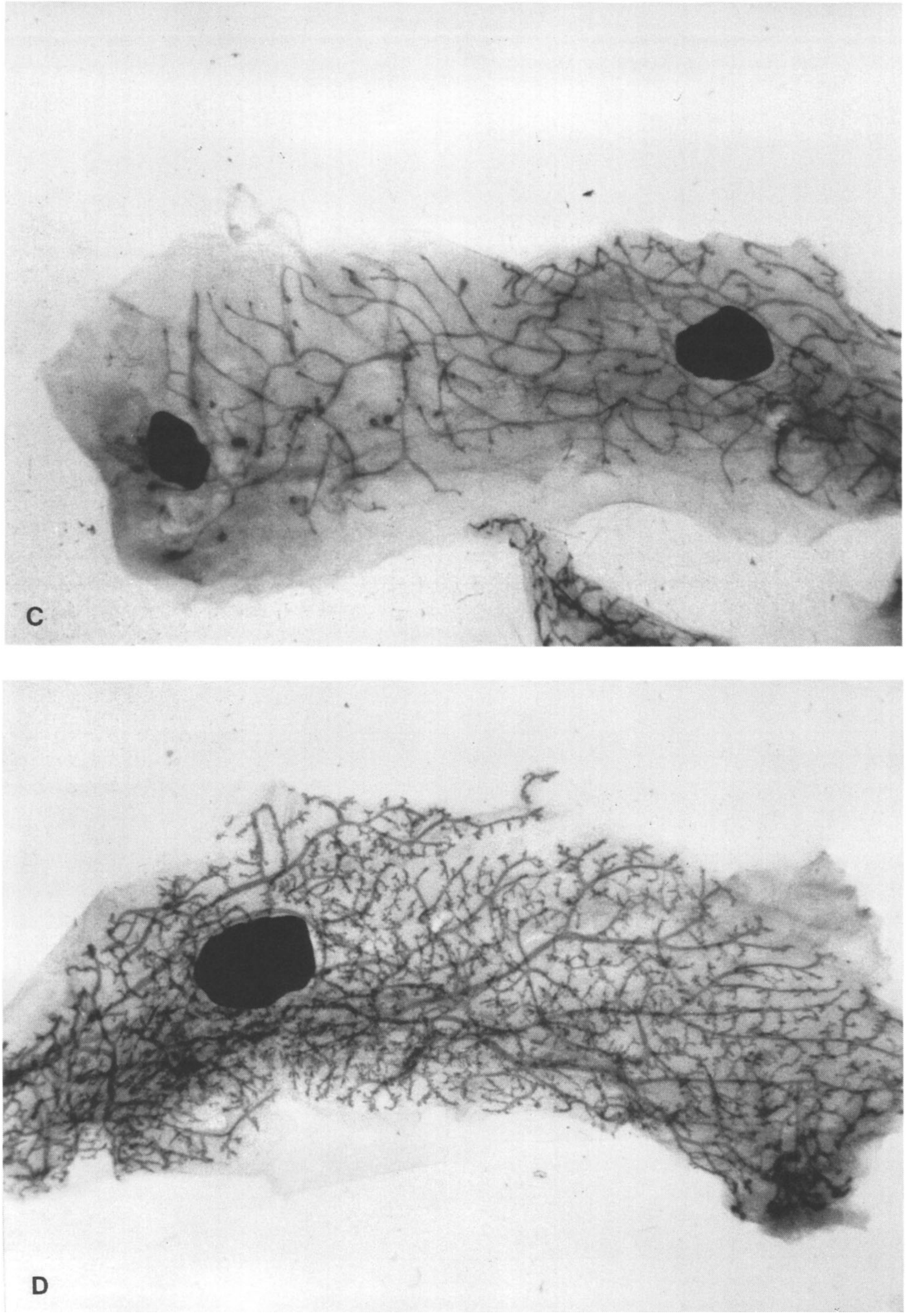

FIG. 2—*Continued.*

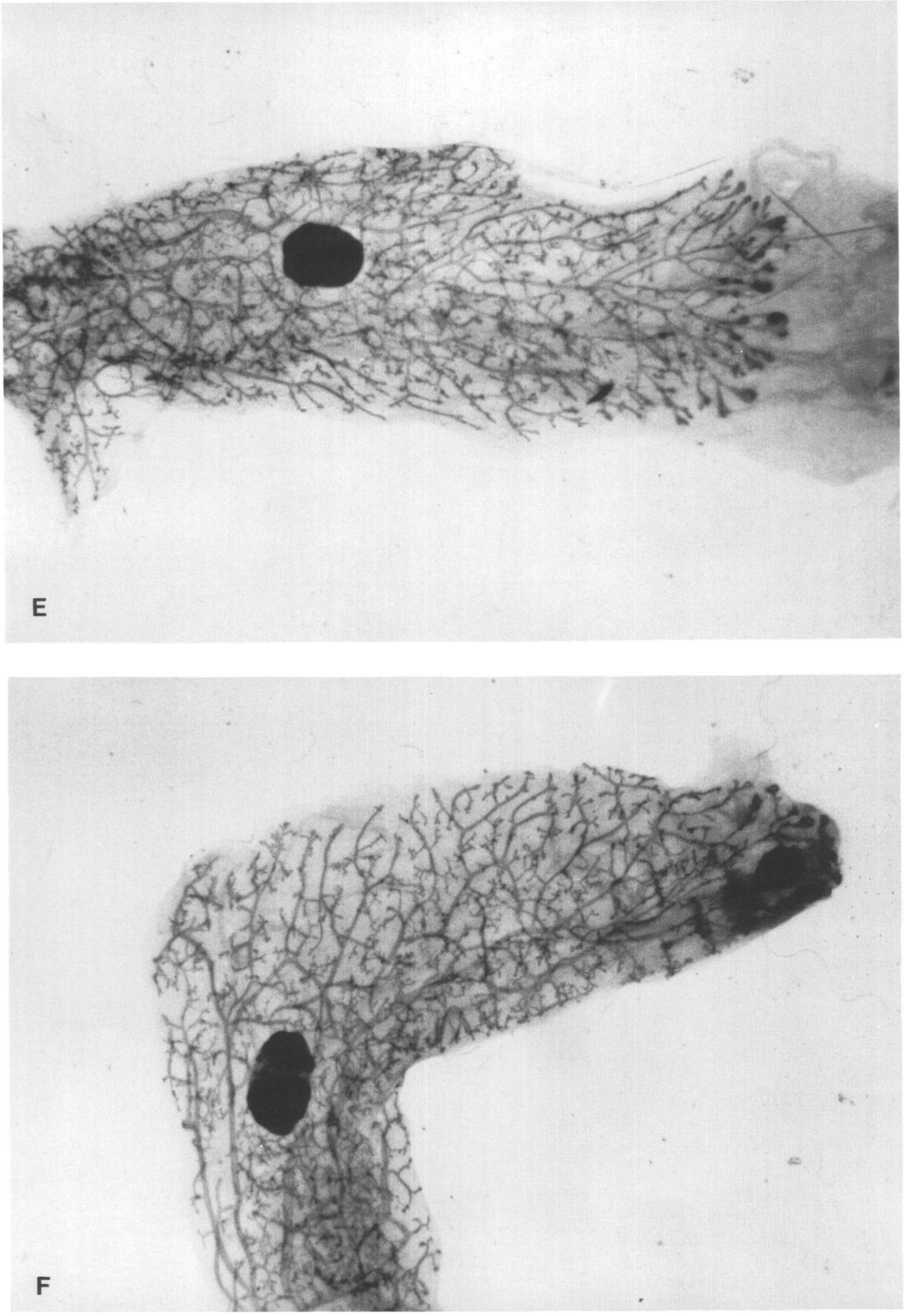

FIG. 2—*Continued.*

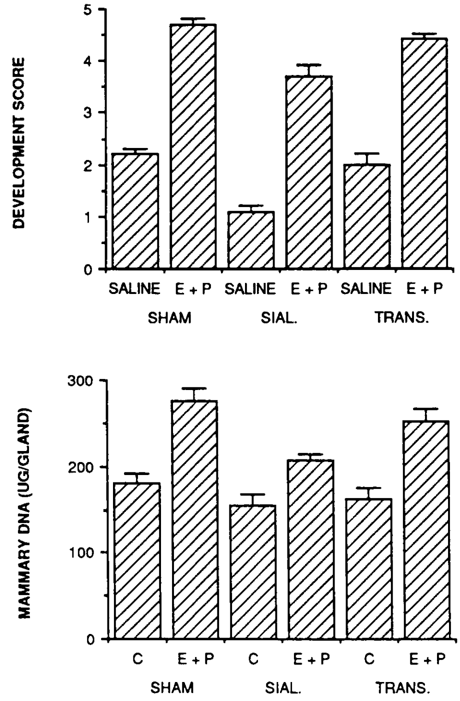

FIG. 3. Mammary gland whole mount development scores and mammary DNA of mice that were sham operated (SHAM), sialoadenectomized (SIAL.), or sialoadenectomized and given subcutaneous salivary gland transplants (TRANS.). The mice were injected daily for 14 days with saline or estradiol + progesterone (E + P). Results are presented as means ± standard error. Sialoadenectomy reduced development score relative to sham operation ($P < 0.05$) in saline- and E + P-treated mice. DNA was reduced by sialoadenectomy in E + P-treated mice ($P < 0.05$). Mammary development score and mammary DNA of mice with salivary transplants were greater than those of sialoadenectomized mice ($P < 0.05$). Trial 1; $n = 10$ mice/group.

the response of mammary DNA to E + P were due largely to differences in mammary DNA among E + P-injected mice. No significant differences in mammary DNA were detected among saline-injected mice that were sham operated, sialoadenectomized, or sialoadenectomized and given salivary transplants ($P > 0.05$).

Trial 2. Because of the difference in body weight observed between sham-operated and sialoadenectomized mice in Trial 1, a second trial was conducted in which mice were fed a liquid diet from weaning until the end of the experiment. Since no differences were observed in mammary DNA of saline-treated mice in Trial 1, all mice in Trial 2 were injected for 14 days with E + P. In this trial, final body weights were 18.0 ± 0.7, 17.8 ± 0.4, and 16.9 ± 0.5 g for sham-operated, sialoadenectomized, and salivary transplant groups, respectively, indicating that the liquid diets largely alleviated the body weight loss due to sialoadenectomy. In this trial, sialoadenectomy also resulted in undetectable serum EGF concentrations in four of six mice. In the two sialoadenectomized mice with detectable serum EGF, it was 1.3 and 1.6 ng/ml (compared to 4.5 ± 0.5 ng/ml for sham-operated mice and 1.4 ± 0.4 ng/ml for mice with salivary transplants). Serum EGF was detectable in 100% of the sham-operated mice and in 100% of the sialoadenectomized mice bearing autografted salivary tissue.

Morphologically, mammae from mice in Trial 2, which were fed liquid diets and injected for 14 days with E + P, were similar to mammae from E + P-treated mice in Trial 1. Mammary gland whole mount development scores were 5.0 ± 0.2 in sham-operated mice. This was reduced to 4.2 ± 0.2 in sialoadenectomized mice ($P < 0.05$). In mice with salivary transplants, mammary gland development scores were intermediate between sham-operated and sialoadenectomized mice (4.8 ± 0.1). Mammary development scores of mice with salivary transplants were greater than those observed in sialoadenectomized mice ($P < 0.05$) but not significantly different than those observed in sham-operated mice. These results were consistent with results obtained with E + P-treated mice in Trial 1.

Mammary DNA levels in Trial 2 followed a pattern similar to that of E + P-treated mice in Trial 1 (Fig. 4). Mammary DNA of sialoadenectomized mice was approximately 81% that of sham-operated mice (255 ± 11 μg/gland in sham-operated mice vs 182 ± 14 μ/gland in sialoadenectomized mice, $P < 0.05$). Mammary DNA of mice with salivary transplants was 228 ± 16 μg/gland, which was not significantly different than that observed in sham-operated mice. The mammary DNA of mice with salivary transplants was not different than sialoadenecto-

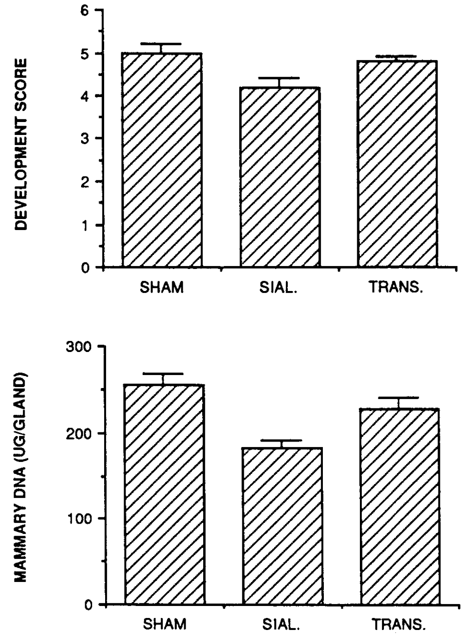

FIG. 4. Mammary gland whole mount development scores and mammary DNA of mice that were sham operated (SHAM), sialoadenectomized (SIAL.), or sialoadenectomized and given subcutaneous transplants of salivary tissue (TRANS.). The mice were treated for 14 days with estradiol + progesterone. Results are presented as means ± standard error. Both measures were reduced by sialoadenectomy ($P < 0.05$). Salivary transplants increased development score ($P < 0.05$) and DNA ($P < 0.10$) in mammary glands of sialoadenectomized mice compared to that of sialoadenectomized mice. Trial 2; $n = 6$ mice/group.

mized mice at $P < 0.05$, but was different at $P < 0.10$ (two-sided test).

Trial 3. In Trial 3, body weights were 18.3 ± 0.7 g for sham-operated mice, 16.8 ± 0.7 g for sialoadenectomized mice, and 17.2 ± 0.5 for sialoadenectomized mice treated with EGF, indicating no significant effect of sialoadenectomy on body weight in mice fed liquid diets. Serum EGF concentration was reduced to undetectable levels by sialoadenectomy (from 3.4 ± 0.7 ng/ml in sham-operated mice) in five of six mice. Serum EGF was 3.4 ± 0.7 ng/ml in sham-operated mice and 0.9 ± 0.03 ng/ml in sialoadenectomized, EGF-treated mice; EGF was detectable in 100% of the mice in these groups.

As in Trials 1 and 2, mammary whole mount development score was reduced by sialoadenectomy (from 5.1 ± 0.1 in controls to 4.3 ± 0.2 in sialoadenectomized mice, $P < 0.05$, Fig. 5). EGF treatment resulted in mean mammary gland development score of 4.8 ± 0.02, which was greater than that observed in sialoadenectomized mice ($P < 0.05$) but not different from that observed in sham-operated mice. As observed in Trials 1 and 2, sialoadenectomy reduced mammary DNA by about 28% relative to sham-operated mice (238 ± 15 μg/gland on controls vs 171 ± 9 μg/gland in sialoadenectomized mice, $P < 0.05$, Fig. 5). EGF resulted in mammary DNA of 211 ± 10 μg/gland, which was greater than that observed in sialoadenectomized mice ($P < 0.05$) but not different from that observed in sham-operated mice.

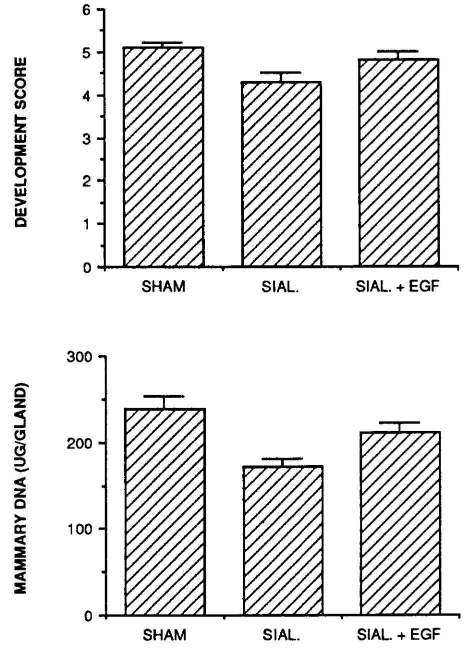

FIG. 5. Mammary whole mount development scores and mammary DNA of mice that were sham operated (SHAM), sialoadenectomized (SIAL.), or sialoadenectomized and injected with epidermal growth factor (SIAL. + EGF). The mice were injected for 14 days with estradiol + progesterone. Sialoadenectomy reduced both measures ($P < 0.05$). Results are presented as means ± standard error. EGF increased development score and DNA in mammary glands from sialoadenectomized mice compared to sialoadenectomized mice ($P < 0.05$). Trial 3; $n = 6$ mice/group.

Discussion. Results of these studies provide evidence that the mouse submandibular salivary gland produces, *in vivo*, mammogenic factors; one such factor may be EGF. These findings are consistent with those of Okamoto and Oka (7), who observed that pregestational sialoadenectomy of mice reduced mammary gland size during subsequent lactation. Their results also suggested that EGF was the mammogenic factor produced by the submandibular salivary gland, as EGF treatment alleviated the effects of sialoadenectomy. This suggestion is consistent with *in vitro* studies, suggesting that EGF increases growth of mammary epithelia (6, 11–13).

Mammary gland receptors for EGF have been shown to increase during early pregnancy (up to about 10 days in mice) and then decline (5), suggesting that the mammary gland is more sensitive to EGF during periods of rapid mammary gland growth (although enhanced sensitivity is not a necessary result of increased receptor content). Recent results (14) indicated that ovarian steroids (estrogen and progesterone) increased a tyrosine phosphorylating activity (possibly EGF receptor) in mouse mammary tissue, suggesting that the mammary EGF receptor is regulated, at least in part, by ovarian steroids. These findings are consistent with those of Vonderhaar (15), who observed that estrogen and progesterone increased the EGF receptor in immature mouse mammary tissue. In light of the present findings, this regulation may be of considerable functional significance, since EGF appears to be required for an optimal mammogenic response to ovarian steroids.

The effect of sialoadenectomy on mammary gland growth appeared to be due to hormonal rather than nutritional factors. In the first trial, in which mice were not fed liquid diets, sialoadenectomized mice were 7% lighter than sham-operated mice ($P < 0.10$). Subcutaneous transplantation of salivary tissue nearly completely restored mammary gland development while body weight still remained less than controls. This restoration of mammary gland developed may have been due to EGF secretion by the transplanted mammary tissue, as serum EGF was more readily detected in this group than in sialoadenectomized mice. However, serum EGF concentrations were typically lower in mice with salivary transplants than in sham-operated mice. In addition, E + P did not increase serum EGF in mice with salivary transplants, as it did in sham-operated mice. These data suggest that some EGF may be needed for normal mammary hormone responsiveness, but that the increase observed upon E + P treatment may not be necessary for normal mammogenesis.

When mice were fed a liquid diet from weaning (Trials 2 and 3), the body weights of controls and sialoadenectomized mice were not significantly different, but sialoadenectomy still decreased mammary gland development. Such results do not necessarily indicate the complete lack of a nutritional difference between sham-operated and sialoadenectomized mice. However, mammary gland development was restored by salivary gland transplants and by EGF injections. These studies would suggest, therefore, a mammogenic endocrine role for the submandibular salivary gland, possibly mediated via EGF.

An important consideration in interpreting the results of these studies is whether sialoadenectomy affected circulating levels of EGF. Early studies indicated that sialoadenectomy did not decrease basal serum EGF concentrations, but did eliminate an α-adrenergic-induced increase in serum EGF (16). However, more recent reports indicate that serum EGF is undetectable in sialoadenectomized mice (3, 7, 17). Results of our study also indicate that sialoadenectomy reduces serum EGF concentrations to undetectable levels in some, but not all, mice. The lack of complete reduction of serum EGF levels in all our sialoadenectomized mice would suggest that EGF is being produced at sites other than the submandibular salivary gland. Interestingly, E + P injections increased serum EGF in sham-operated mice, but not in sialoadenectomized mice, with or without salivary transplants. This observation provides evidence that only an intact submandibular salivary gland is responsive to induction of EGF secretion by ovarian steroids. Our results confirm and extend the study of Kurachi and Oka (3), who reported that serum EGF levels are increased during pregnancy in mice. However, the absolute serum EGF concentrations in our study was higher than that found by Kurachi and Oka (3). The serum EGF level in our study was similar to that reported by Byyny *et al.* (16). More recent studies by several laboratories (3, 18) suggest that serum EGF concentrations may be considerably lower than reported by Byyny *et al.* (16). Therefore, the possibility exists that the apparent decrease in serum EGF we observed was due to a cross-reactive substance in the serum of salivary gland origin.

In summary, our results are consistent with an *in vivo* model of mammary gland development that includes EGF as a hormonal mediator of mammogenesis. These results do not, however, eliminate the possibility that the submandibular salivary gland produces mammogenic factors other than or in addition to EGF.

---

1. Cohen S, Savage CR Jr. Recent studies on the chemistry and biology of epidermal growth factor. Recent Prog Horm Res **30**:551–574, 1974.
2. Ances IG. Serum concentration of epidermal growth factor in human pregnancy. Amer J Obstet Gynecol **115**:357–362, 1973.
3. Kurachi H, Oka T. Changes in epidermal growth factor concentration of submandibular gland, plasma and urine of normal and sialoadenectomized female mice during various reproductive stages. J Endocrinol **106**:197–202, 1985.
4. Edery M, Pang K, Larson L, Colsi T, Nandi S. Epidermal growth factor receptor levels in mouse mammary glands in various physiological states. Endocrinology **117**:405–411, 1985.
5. Vonderhaar BK, Tang E, Lyster RR, Nascimento MCS. Thyroid hormone regulation of epidermal growth factor receptor levels in mouse mammary glands. Endocrinology **119**:580–585, 1986.
6. Taketani Y, Oka, T. Epidermal growth factor stimulates cell proliferation and inhibits functional differentiation of mouse mammary epithelial cells in culture. Endocrinology **113**:871–877, 1983.
7. Okamoto S, Oka T. Evidence for physiological function of epidermal growth factor: Pregestational sialoadenectomy of mice decreases milk production and increases offspring mortality during lactation period. Proc Natl Acad Sci USA **81**:6059–6063, 1984.
8. Welsch CW, Gribler C. Prophylaxis of spontaneously developing mammary carcinoma in C3H/HeJ female mice by suppression of prolactin. Cancer Res **33**:2939–2946, 1973.
9. Burton KA. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J **62**:315–323, 1956.
10. Gill JL. Design and Analysis of Experiments in the Animal and Medical Sciences. Ames, IA, Iowa State University Press, Vol. 1, 1978.
11. Stoker MGP, Pigott D, Taylor-Papadimitriou J. Response to epidermal growth factor of cultured human mammary epithelial cells from benign tumors. Nature (London) **264**:764–765, 1976.
12. Tonelli QJ, Soroff S. Epidermal growth factor requirement for development of cultured mammary gland. Nature (London) **285**:250–252, 1980.
13. Yang J, Guzman R, Richards J, Imagawa W, McCormick K, Nandi S. Growth factor- and cyclic nucleotide-induced proliferation of normal and malignant mammary epithelial cells in primary culture. Endocrinology **107**:35–41, 1980.
14. Sheffield LG, Aylsworth CF, Welsch CW. Cyclic neucleotides and protein phosphorylation in mouse mammary glands: Effects of estrogen and progesterone administration in vivo. Proc Soc Exp Biol Med **185**:283–290, 1987.
15. Vonderhaar BK. Hormones and growth factors in mammary gland development. In: Veneziale CM, Ed. Control of Cell Growth and Proliferation. New York, Van Norstand–Reinhold, pp. 11–33, 1985.
16. Byyny R, Orth DN, Cohen S, Doynt E. Epidermal growth factor: Effects of androgens and adrenergic agents. Endocrinology **95**:776–783, 1974.
17. Tsutsumi O, Kurachi H, Oka T. A physiological role of epidermal growth factor in male reproductive function. Science **233**:975–978, 1986.
18. Perheentupa J, Lakshmanan J, Hoath SB, Fisher DA. Hormonal modulation of mouse plasma concentration of epidermal growth factor. Acta Endocrinol **107**:571–576, 1984.

Received May 29, 1987. P.S.E.B.M. 1987, Vol. 186.
Accepted September 2, 1987.