[CANCER RESEARCH 39, 239-243, January 1979]
0008-5472/79/0039-0000$02.00

Effects of Epidermal Chalone and Epidermal Growth Factor on a Transplantable Epidermal Carcinoma (Hewitt) of the Mouse *in Vivo*

Stefan Bertsch[1] and Friedrich Marks

German Cancer Research Center, Institute of Biochemistry, Im Neuenheimer Feld 280, D-6900 Heidelberg, Germany

ABSTRACT

The effects of chalone-containing skin preparations and of epidermal growth factor on the proliferative activity of a transplantable nonkeratinizing epidermal carcinoma (Hewitt) of the mouse have been studied *in vivo*. Whereas crude skin extracts suppressed DNA labeling (probably due to cytotoxic effects), highly enriched epidermal G_1 chalone was found to inhibit neither DNA synthesis nor tumor growth. Consequently, repeated injections of the factor did not prolong the survival rate of tumor-bearing animals.

Extracts made from tumor tissue did not show any G_1 chalone activity when injected into normal mice, whereas extracts from normal mouse epidermis exhibited a strong inhibitory effect on epidermal DNA labeling. Despite being resistant to epidermal G_1 chalone, the carcinoma was found to be susceptible to the antimitotic effects of skin extracts which are most probably due to the epidermal G_2 chalone.

A single i.p. injection of epidermal growth factor caused a doubling of DNA labeling in the transplanted tumor.

These observations are consistent with the hypothesis that epidermal G_1 chalone controls only the proliferation of epidermal "stem cells" in a rather advanced stage of differentiation, whereas epidermal G_2 chalone as well as epidermal growth factor appear to affect more primitive cell types.

INTRODUCTION

When skin or epidermis extracts are injected into mice, DNA synthesis and mitotic activity are inhibited in the epidermis of these animals. These effects are due to 2 endogenous inhibitors of cell proliferation, called G_1 and G_2 chalones in order to indicate their points of attack in the cell cycle (11, 18). Although the physiological role of these factors is still a matter of dispute, they have been proposed to be of some value for a tissue-specific control of tumor growth (4–6). Indeed, several promising observations have been published (7, 8, 10, 15). However, all these experiments were hampered by the use of rather crude skin extracts instead of purified chalone as well as by the fact that it was mostly the effect of G_2 chalone that was measured; no conclusive data are as yet available for G_1 chalone. Since we have recently succeeded in purifying epidermal G_1 chalone from pigskin (20), we wish now to present some data on the effect of this compound on the growth of a highly dedifferentiated transplantable squamous carcinoma of the mouse. For comparison, some preliminary experiments regarding the effects of 2 other control factors, *i.e.*, epidermal G_2 chalone and EGF,[2] were made.

MATERIALS AND METHODS

Animals. Female and male adult WHT mice were used for tumor transplantation. Chalone assays were performed with female NMRI mice (7 to 8 weeks old). The animals were fed *ad libitum* and kept under an artificial day-night rhythm (19). Four days prior to the experiments, the back skin of the mice was shaved, and only those animals were used which did not show a regrowth of hair.

Chemicals. [^{3}H]Thymidine (specific activity, 6.7 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Vincristine was purchased from Lilly, Giessen, Germany. As epidermal chalone preparation, a 55 to 72% ethanol precipitate and a 72 to 81% ethanol precipitate of an aqueous extract from pigskin (Codes CH 1332 and CH 1333) were used. These fractions were kindly supplied by Dr. W. Hondius-Boldingh (N. V. Organon, Oss, The Netherlands). EGF was prepared from mouse salivary glands according to the procedure of Savage and Cohen (26).

Partial Purification of Epidermal G_1 Chalone. Epidermal G_1 chalone was partially purified from a 55 to 72% ethanol precipitate of an aqueous extract made from defatted and lyophilized pigskin powder (13). The ethanol fraction (1 g) was dissolved in 300 ml water and ultrafiltrated with 5 × 1 liter water through a Diaflo XM-300 membrane filter (Amicon). Then the pH of the residue was adjusted to 5 with diluted acetic acid, and the solution was allowed to stand at 4° for 2 days. After centrifugation, 160 to 200 mg of a brownish sediment were obtained which contained most of the chalone activity. This material was dissolved in 20 ml 0.1 M Tris-HCl buffer (pH 7.4) and incubated with 0.4% dithiothreitol at room temperature and under a nitrogen atmosphere for 20 hr (to avoid microbial growth, a few drops of toluol and 0.02% NaN_3 were added). Then iodoacetamide (1.5%) was added. After 2 hr, a further addition of dithiothreitol (3%) was made, and the mixture was kept at room temperature for a further 2 hr. Afterward, the precipitate, which contained almost no chalone activity, was collected by centrifugation and discarded. The supernatant was dialyzed against several liters of water at 4° overnight and lyophilized. The lyophilysate was then dissolved in a small volume of 7 N urea in 0.05 M Tris-acetate buffer (pH 7.4) containing 0.02% sodium azide and placed on a column (3 x 12 cm) of DEAE-cellulose (Whatman DE 23). The column was eluted with 10 ml 7 M urea in 0.05 M Tris-acetate buffer (pH 7.4) and then with 200 ml of a 0 to 0.5 M NaCl gradient in the same solution. Finally, the column was washed with 1 M NaCl in urea-buffer solution. Monitoring the absorbance at 280 nm, 2 large peaks were obtained, with the chalone activity being entirely concentrated in the second peak. The corresponding opalescent fractions were collected, dialyzed against water, and lyophilized (yield, 30 to 40 mg).

[1] To whom requests for reprints should be addressed.
[2] The abbreviation used is: EGF, epidermal growth factor.
Received June 16, 1978; accepted September 27, 1978.

When injected i.p. into an adult mouse, 3 to 5 μg of this material were sufficient to depress epidermal DNA synthesis *in vivo* about 50%. This corresponds approximately to a 20,000-fold enrichment of chalone as compared with the original lyophilized pigskin powder.

Treatment of Animals. [^{3}H]Thymidine, skin and tumor extracts, vincristine, and EGF were dissolved in 0.9% NaCl solution (0.2 to 0.3 ml) and injected i.p. Control animals received only 0.9% NaCl solution.

Tumor Transplantation. Tumor-bearing WHT mice (a gift of Dr. H. B. Hewitt, Gray Laboratory, Mount Vernon Hospital, Northwood Middlesex, Great Britain) were killed by cervical dislocation, and tumors were dissected under aseptic conditions. The tumors were mechanically macerated and suspended in 0.9% NaCl solution. The tumor cell suspension was injected s.c. under the back skin of WHT mice. Two weeks later, the tumors had developed at the site of inoculation, and the animals were available for the experiments.

Pulse-Labeling and Preparation of Tumor and Epidermal DNA. DNA was pulse-labeled by an i.p. [^{3}H]thymidine injection of 1.5 μCi/g body weight. One hr later, the animals were sacrificed, and tumor tissue or back skin was dissected. Epidermis was separated from dermis by scraping with a scalpel. Tumor tissue or epidermis was then homogenized, and the DNA was isolated, determined, and assayed for specific radioactivity as described previously (17).

Preparation of Tumor Extract. Pieces of tumor tissue free of necrotic components were frozen in liquid nitrogen and homogenized in an electric mill. The resulting powder was stirred in water for 30 min. From this suspension, a 105,000 × *g* supernatant was made and was used in the experiments. As a control, an epidermal extract was prepared. For this purpose, mouse back skin was dissected, and epidermis was separated from dermis by incubation in 1% acetic acid at 4° overnight. The epidermis was homogenized and extracted as described for tumor tissue.

Determination of Mitotic Activity. Vincristine (25 μg) was injected i.p. into tumor-bearing mice. After 4 hr, the animals were killed by cervical dislocation. The tumors were dissected and fixed in 10% formalin; 5-μm sections were made and stained with hematoxylin-eosin. From these sections, 8 visual fields per tumor were photographed, and the metaphase figures were counted. These visual fields represent about 4900 nucleated cells.

RESULTS

Effects of Epidermal G_1 Chalone on Tumor Growth. To find out whether or not epidermal G_1 chalone had an effect on DNA synthesis of tumor cells, skin fractions were injected i.p. into tumor-bearing mice, *i.e.*, 5 mg of pigskin preparation (55 to 72% ethanol precipitate of an aqueous skin extract; see Ref. 13) or 350 μg of a highly enriched chalone fraction corresponding to 10 mg of ethanol precipitate (19). This is approximately 50- to 100-fold the dose sufficient to inhibit DNA synthesis in normal mouse epidermis *in vivo* for 50 to 70% (19). The DNA synthesis in tumor tissue was measured by means of pulse-labeling with [^{3}H]thymidine *in vivo* at different time intervals after the chalone treatment. As Chart 1 shows, the crude skin extract led to a depression of DNA labeling between 6 and 9 hr, whereas after 12 to 15 hr (when in normal epidermis the effect reaches a maximum; see Ref. 19) no significant inhibition could be seen. During this treatment, the animals showed symptoms of an intoxication obviously due to the extract applied. No such illness was seen after application of purified chalone preparation, and only a slight, hardly significant inhibition of DNA labeling occurred which was followed by a moderate stimulation.

In spite of these different effects on tumor tissue, both chalone preparations exhibited a strong inhibition on epidermal DNA labeling when injected into normal WHT mice (Table 1). To confirm these results, the effect of enriched G_1 chalone on tumor growth *in vivo* was investigated. For this purpose, daily injections of 75 μg chalone per animal were made over a period of 13 days beginning at the day of tumor inoculation. At Days 1 and 13, the dose was doubled. The dose per day was approximately 10- to 20-fold that sufficient to depress DNA synthesis in normal epidermis for 50 to 70%. As a measure of an inhibitory effect, the survival rate of the animals was taken. As shown in Chart 2, G_1 chalone treatment could not delay the development of the tumor. Thirteen days after the last chalone injection, only 3 of 10 animals were still alive in the control group as well as in the group of chalone-treated mice.

"Chalone Activity" of Tumor Extracts. The Hewitt carcinoma used in the experiments is a highly dedifferentiated tumor which during subsequent passages has lost its ability to keratinize (H. B. Hewitt, personal communication). Since according to both theoretical considerations (28) and sev-

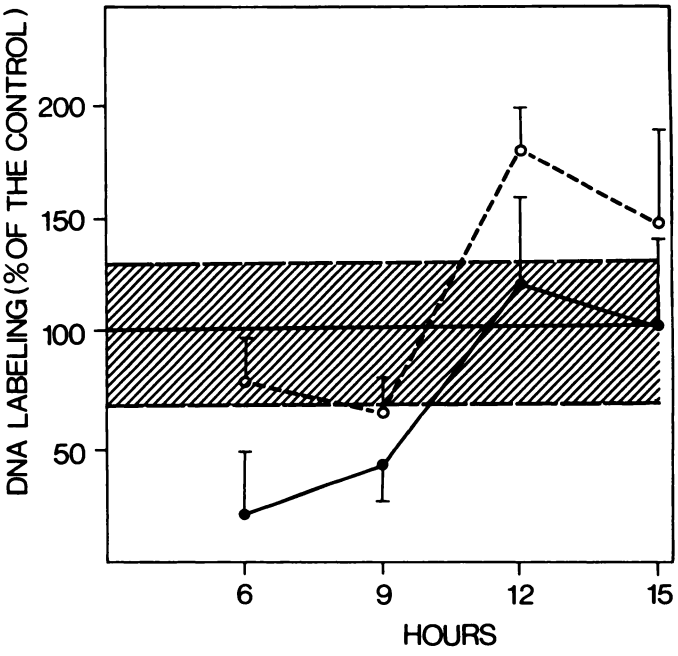

Chart 1. Effects of an ethanol precipitate of pigskin extract and of a highly enriched G_1 chalone fraction on DNA labeling in a nonkeratinizing epidermal carcinoma (Hewitt). The animals were given i.p. injections of 0.2 ml 0.9% NaCl solution (controls) or 5 mg ethanol precipitate (●) or 350 μg highly enriched fraction (○) in 0.9% NaCl solution at zero time and killed at the times indicated. One hr prior to sacrifice, 1.5 μCi of [^{3}H]thymidine per g body weight were injected i.p. Each *point* represents the mean value of experiments with at least 20 animals. *Bars*, S.D.; *shaded area*, average S.D. of the controls ($N > 80$).

Table 1

Effect of an ethanol precipitate of pigskin extract and of a highly enriched G_1 chalone fraction on DNA labeling in normal mouse epidermis

The animals were given i.p. injections of 0.2 ml of 0.9% NaCl solution (controls) or 5 mg ethanol precipitate or 350 μg highly enriched fraction in 0.9% NaCl solution at zero time. After 14 hr, 1.5 μCi of [^{3}H]thymidine per g body weight were injected i.p. After an additional hr, the animals were killed.

	cpm/μg DNA	% of the control
Control	27 ± 3[a]	100 ± 12
Ethanol precipitate (5 mg)	8 ± 2	30 ± 8
Highly enriched fraction (350 μg)	6 ± 1	22 ± 4

[a] Mean ± S.D.; $N = 15$.

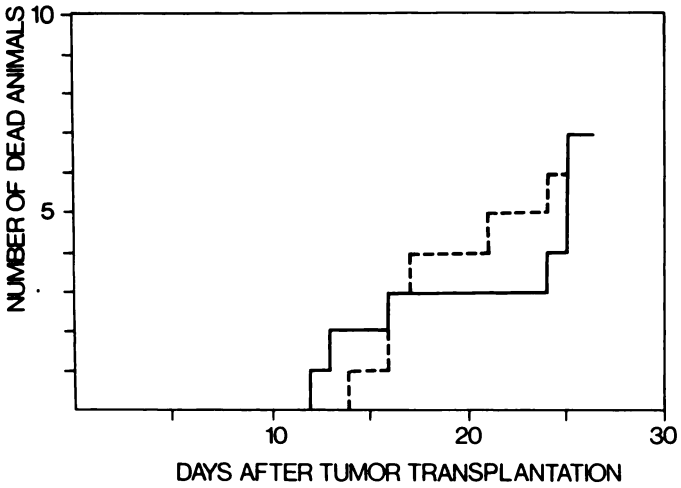

Chart 2. Effect of highly enriched G_1 chalone fraction on the survival rate of tumor-bearing animals. The tumor (Hewitt) was inoculated s.c. at zero time. Daily i.p. injections of 75 μg of the chalone preparation (----) in 0.2 ml 0.9% NaCl solution were made over a period of 13 days beginning at the day of the tumor inoculation. At Days 1 and 13, the dose was doubled. The control animals (——) received only 0.9% NaCl solution. At Day 26, the remaining 3 animals in each group were killed.

eral observations (22)[3] epidermal G_1 chalone is thought to be synthesized in the course of cell differentiation (*i.e.*, keratinization), it was interesting to know whether or not tumor tissue contains "G_1 chalone activity." For this purpose, an aqueous extract was prepared and tested for inhibitory activity. This was performed by measuring epidermal DNA labeling *in vivo* 15 hr after an i.p. injection of 5 mg extract (dry weight) into normal NMRI mice. As a control, an extract was made under corresponding conditions from pure epidermis of WHT mice and tested for activity. While the tumor extract did not exhibit any effect on DNA labeling, the epidermis extract led to a pronounced depression of thymidine incorporation (Table 2).

"G_2 Chalone Effects" on Tumor Cell Proliferation. When injected into normal adult mice, skin extracts specifically inhibit both epidermal DNA synthesis (G_1 chalone activity) and epidermal mitotic rate (G_2 chalone activity). To evaluate a possible effect of G_2 chalone on tumor cell proliferation, a pigskin preparation [72 to 81% ethanol precipitate of an aqueous skin extract (13)] which, besides some G_1 chalone activity (18), predominantly exhibits G_2 chalone activity (13) was injected i.p. into tumor-bearing mice together with vincristine as a stathmokinetic agent. After 4 hr, mitotic figures were counted in the tumor tissue. As shown in Chart 3, a 75% depression of mitotic activity in the tumor had occurred. Since in contrast to epidermal G_1 chalone the G_2 chalone is heat labile, a control experiment was carried out in which the skin fraction was heated in a boiling water bath for 10 min prior to injection. After this treatment, the extract had completely lost its antimitotic activity (Chart 3). This and the observation that the animals treated with the nondenatured extract did not show any symptoms of intoxication may be taken as evidence that the inhibitory effect observed was due to epidermal G_2 chalone instead of to more unspecific or cytotoxic factors.

Effect of EGF on Tumor Cell Proliferation. Recently, EGF, a peptide hormone isolated from mouse salivary

Table 2

Effect of an extract from a nonkeratinizing epidermal carcinoma (Hewitt) and from normal mouse epidermis on DNA labeling in mouse epidermis in vivo

For further details, see legend to Table 1.

	cpm/μg DNA	% of the control
Control	34 ± 11[a]	100 ± 33
Tumor extract (5 mg)	34 ± 12	100 ± 37
Epidermis extract (5 mg)	12 ± 2	47 ± 7

[a] Mean ± S.D.; $N = 15$.

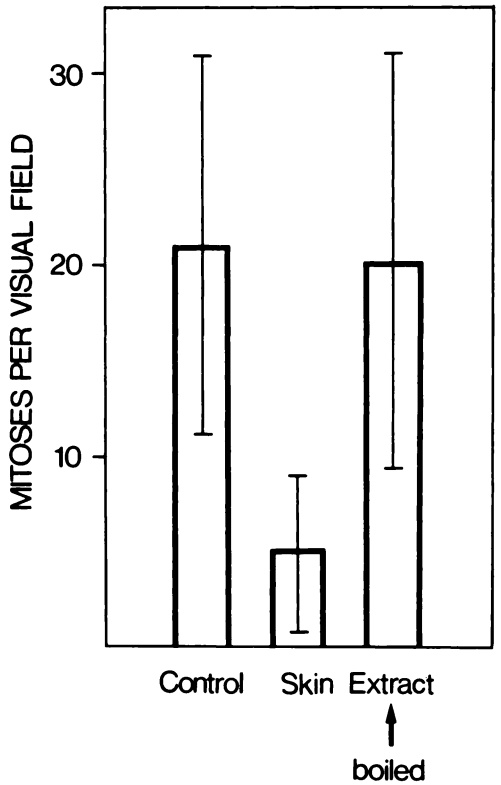

Chart 3. Effect of a G_2 chalone-containing pigskin extract on the mitotic activity of a nonkeratinizing epidermal carcinoma (Hewitt). Four hr before sacrifice, the animals received an i.p. injection of 25 μg vincristine per animal in 0.2 ml 0.9% NaCl solution (controls) together with 5 mg normal or boiled pigskin extract. The mitotic activity is expressed as the number of mitoses per visual field. *Bars*, S.D.

[3] S. Bertsch and F. Marks. Appearance of Growth-inhibiting Activity (G_1 Chalone) during Ontogenetic Development of Rat and Chick Epidermis *in Vivo* and *in Vitro*, manuscript in preparation.

glands, has been shown to enhance the growth of undifferentiated embryonic epidermis (2, 9) as well as of epidermal tumors generated by polycyclic hydrocarbons (23, 25) but not of epidermis of mature animals (9). A similar observation was made regarding the Hewitt carcinoma *in vivo*. Purified EGF was injected i.p. into tumor-bearing mice in a dose of 3 μg/g body weight. After 15 hr, DNA labeling was measured according to the method described above. Whereas in tumor tissue a 200% stimulation was observed, the hormone did not exhibit any effect on the epidermis of normal mice (Chart 4). Supplies of EGF were inadequate to study the kinetics of the stimulatory effect.

DISCUSSION

The results presented here demonstrate that DNA labeling in a highly dedifferentiated squamous cell carcinoma of the mouse is inhibited by injections of crude skin extract. This effect is probably due to cytotoxicity rather than being specific, since purified epidermal G_1 chalone apparently does not show a significant inhibitory activity. In accordance with this observation, the factor was found to be unable to delay tumor growth or prolong the survival time of tumor-bearing animals even when applied in great excess.

Not only are these results consistent with similar observations of Elgjo and Hennings (10), but they also confirm the idea that epidermal G_1 chalone controls exclusively a rather advanced stage of epidermal stem cell proliferation, perhaps only the last cell division(s) prior to the postmitotic period (21). This was originally indicated by experiments with newborn animals (3) as well as with adult epidermis exposed to a "hyperplastic transformation" (1, 17).

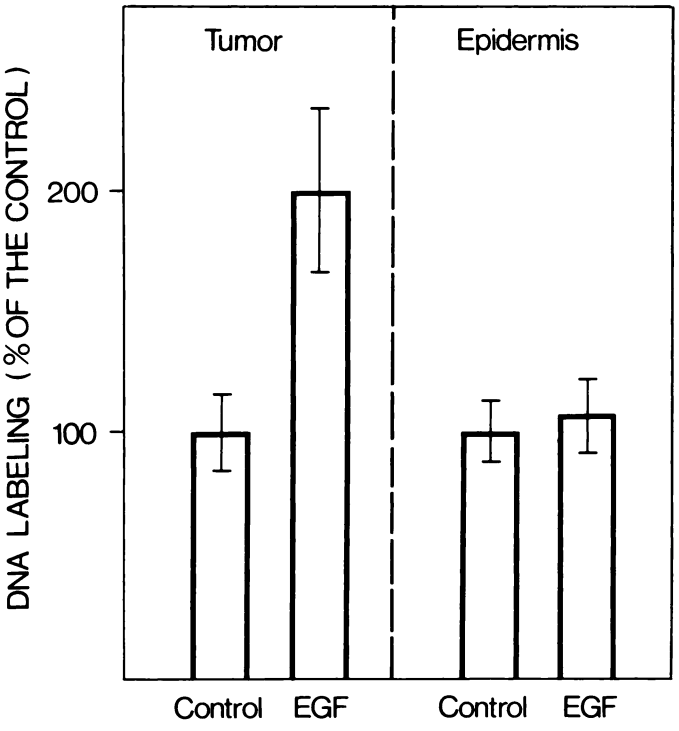

Chart 4. Effect of EGF on DNA labeling in a nonkeratinizing epidermal carcinoma (Hewitt) and in normal mouse epidermis. EGF (3 μg/g body weight) in 0.2 ml 0.9% NaCl solution were injected i.p. Control animals received 0.9% NaCl solution. Fourteen hr after treatment, 1.5 μCi of labeled thymidine per g body weight were injected i.p. After an additional hr, the animals were killed. Each *column* represents the mean value of experiments with at least 15 animals. *Bars*, S.D.

Obviously, the cells of the Hewitt carcinoma which do not show any signs of keratinization never reach this stage of differentiation, being therefore nearly resistant to G_1 chalone inhibition. Apparently, they also do not produce the factor. In these respects, they behave like epidermis in a rather early stage of embryonic development.[3]

The slight stimulatory effect of epidermal G_1 chalone on DNA labeling in tumor tissue cannot now be explained. However, a similar observation was made after chalone treatment of newborn mice (3). Possibly, the preparation still contains some stimulatory component beside the chalone, or both the inhibitory and the stimulatory activities are due to the factor depending on the target tissue.

Regarding epidermal G_2 chalone, the situation is obviously quite different. There are several reports indicating an inhibitory effect of this factor in neonatal (19) as well as on hyperplastically transformed (17) and neoplastic epidermis (15). The experiment reported here is of course hampered by the fact that only a rather crude G_2 chalone preparation was used. However, the apparent nontoxicity and the heat sensitivity may be taken as evidence that the antimitotic effect on the tumor was due to the chalone. This conclusion is confirmed by recent results of Korsgaard *et al.* (15) showing a strong inhibitory action of purified G_2 chalone on squamous carcinoma cells of the human respiratory tract *in vitro* which was independent of the degree of differentiation of the tumor (15). Although highly cell line specific, epidermal G_2 chalone thus obviously controls the proliferation of more "primitive" epidermal cell types. It may be, therefore, a much better candidate for cancer control than epidermal G_1 chalone is. Whereas the intact epidermis of adult animals (9) apparently does not respond to EGF, the growth of embryonic (2, 9) as well as of wounded epithelium (27) is considerably enhanced both *in vivo* and *in vitro*. Furthermore, the development of epidermal tumors has been reported to be stimulated by EGF (23, 25) which is in accordance with the experiment presented here. Finally, even the proliferation of nonepidermal cells is accelerated by this growth factor (12, 24, 29). Thus, EGF probably may be considered to be a stimulatory hormone for more primitive stem cells such as those existing in embryonic tissue, wounds, and tumors.

REFERENCES

1. Bertsch, S., Csontos, K., Schweizer, J., and Marks, F. Effect of Mechanical Stimulation on Cell Proliferation in Mouse Epidermis and on Growth Regulation by Endogenous Factors (Chalones). Cell Tissue Kinet., *9:* 445–457, 1976.
2. Bertsch, S., and Marks, F. Effect of Fetal Calf Serum and Epidermal Growth Factor on DNA Synthesis in Explants of Chick Embryo Epidermis. Nature, *251:* 517–518, 1974.
3. Bertsch, S., and Marks, F. Lack of an Effect of Tumor-promoting Phorbol Esters and Epidermal G_1 Chalone on DNA Synthesis in the Epidermis of Newborn Mice. Cancer Res., *34:* 3283–3288, 1974.
4. Bullough, W. S. Mitotic and Functional Homeostasis: A Speculative Review. Cancer Res., *25:* 1683–1727, 1965.
5. Bullough, W. S. The Control of Epidermal Thickness. Brit. J. Dermatol., *87:* 187–189, 347–354, 1972.
6. Bullough, W. S., and Deol, J. U. R. The Pattern of Tumor Growth. Symp. Soc. Exptl. Biol., *25:* 255–275, 1971.

7. Bullough, W. S., and Deol, J. U. R. Chalone-Induced Mitotic Inhibition in the Hewitt Keratinizing Epidermal Carcinoma of the Mouse. European J. Cancer, *7:* 425–431, 1971.
8. Bullough, W. S., and Laurence, E. B. Control of Mitosis in Rabbit V × 2 Epidermal Tumors by Means of the Epidermal Chalone. European J. Cancer, *4:* 587–594, 1968.
9. Cohen, S. Studies on the Mechanism of Action of Epidermal Growth Promoting Factor (EGF). *In:* M. Hamburgh and E. J. W. Burrington (eds.), Hormones in Development, pp. 753–766. New York: Meredith Corp., 1971.
10. Elgjo, K., and Hennings, H. Epidermal Chalone and Cell Proliferation in a Transplantable Squamous Cell Carcinoma in Hamsters. I. *In vivo* Results. Virchows Arch. B Zellpathol., *7:* 1–7, 1971.
11. Hennings, H., Elgjo, K., and Iversen, C. H. Delayed Inhibition of Epidermal DNA Synthesis after Injection of an Aqueous Skin Extract (Chalone). Virchows Arch. B Zellpathol., *4:* 45–53, 1969.
12. Hollenber, M. D., and Cuatrecasas, P. Insulin und Epidermal Growth Factor. J. Biol. Chem., *250:* 3845–3853, 1975.
13. Hondius-Boldingh, W., and Laurence, E. B. Extraction, Purification and Preliminary Characterization of the Epidermal Chalone: A Tissue-specific Inhibitor Obtained from Vertebrate Skin. European J. Biochem., *5:* 191–198, 1968.
14. Iversen, O. H., Bangoo, K. S., and Hansen, K. Control of Epidermal Cell Renewal in the Bat Web. Virchows Arch. B Zellpathol., *16:* 157–179, 1974.
15. Korsgaard, R., Iversen, O. H., Burton, D. R., and Isaksson-Forsén, G. Artunspezifische und reversible Wachstumshemmung durch Chalone in Plattenepithelkarzinomen des Menschen *in vitro*. Z. Krebsforsch., *88:* 217–221, 1977.
16. Krieg, L. Zur Wachstumskontrolle in Mäuseepidermis nach Einwirkung hyperplasiogener und tumorpromovierender Reize. Ph.D. Dissertation, University of Heidelberg, 1975.
17. Krieg, L., Kühlmann, I., and Marks, F. Effect of Tumor-promoting Phorbol Esters and Acetic Acid on Mechanisms Controlling DNA Synthesis and Mitosis (Chalones) and on the Biosynthesis of Histidine-rich Protein in Mouse Epidermis. Cancer Res., *34:* 3135–3146, 1974.
18. Marks, F. Direct Evidence of Two Tissue-specific Chalone-like Factors Regulating Mitosis and DNA Synthesis in Mouse Epidermis. Z. Physiol. Chem., *352:* 1273–1274, 1971.
19. Marks, F. A Tissue-specific Factor Inhibiting DNA Synthesis in Mouse Epidermis. Natl. Cancer Inst. Monograph, *38:* 79–90, 1973.
20. Marks, F. Isolation of an Endogenous Inhibitor of Epidermal DNA Synthesis (G_1 Chalone) from Pig Skin. Z. Physiol. Chem., *356:* 1989–1992, 1975.
21. Marks, F., Bertsch, S., Grimm, W., and Schweizer, J. Hyperplastic Transformation and Tumor Promotion in Mouse Epidermis: Possible Consequences of Disturbances of Endogenous Mechanisms Controlling Proliferation and Differentiation. *In:* T. Sluga, A. Sivak, and R. K. Boutwell (eds.), Carcinogenesis, Vol. 2, pp. 97–116. New York: Raven Press, 1978.
22. Nome, I. Tissue Specificity of the Epidermal Chalones. Virchows Arch. B Zellpathol., *19:* 1–25, 1975.
23. Reynolds, V. H., Boehm, F. H., and Cohen, S. Enhancement of Chemical Carcinogenesis by an Epidermal Growth Factor. Surg. Forum, *16:* 108–109, 1965.
24. Rose, P., Pruss, R. M., and Herschman, H. R. Initiation of 3T3 Fibroblast Cell Division by Epidermal Growth Factor. J. Cellular Physiol., *86:* 593–598, 1975.
25. Rose, P., Stahn, R., Passovoy, D. S., and Herschman, H. Epidermal Growth Factor Enhancement of Skin Tumor Induction in Mice. Experientia, *32:* 913–915, 1976.
26. Savage, C. R., and Cohen, S. Epidermal Growth Factor and a New Derivative. J. Biol. Chem., *247:* 7609–7611, 1972.
27. Savage, C. R., and Cohen, S. Proliferation of Corneal Epithelium Induced by Epidermal Growth Factor. Exptl. Eye Res., *15:* 361–366, 1973.
28. Weiss, R., and Kavanau, J. L. A Model of Growth and Growth-Control in Mathematical Terms. J. Gen. Physiol., *41:* 1–47, 1957.
29. Westermark, B. Density Dependent Proliferation of Human Glia Cells Stimulated by Epidermal Growth Factor. Biochem. Biophys. Res. Commun., *69:* 304–310, 1976.