Epidermal Growth Control Mechanisms, Hyperplasia, and Tumor Promotion in the Skin

Friedrich Marks
Institute of Biochemistry, German Cancer Research Center (Deutsches Krebsforschungszentrum), D-69 Heidelberg, Germany

Summary

In permanently renewing epithelia such as epidermis, the steady state between cell loss and cell gain is thought to be controlled by tissue-own and tissue-specific inhibitors of cell proliferation (chalones). In epidermis two chalone-like factors have been found, a G1 chalone retarding the entry of cells into the phase of DNA replication and a G2 chalone inhibiting the transition of cells from G2 into mitosis. Both factors have been isolated from pigskin; they are probably glycoproteins. In contrast to the G2 chalone, the G1 chalone is resistant to heat and proteolytic enzymes and is most probably produced in keratinizing cells. From the purified G1 chalone a dose of 0.05 to 0.1 μg injected i.p. into an adult mouse is sufficient to depress epidermal DNA synthesis to about 50%. Studies on the response of mouse epidermis to mitogenic stimuli lead to the conclusion that the tissue can react in two different ways:

1. If the stimulus does not involve severe injury, only the flow of cell through the steady state of tissue homeostasis is accelerated (e.g., after gentle skin massage or chemical depilation). In this case hyperplasia is not observed or develops only slowly if the stimulus lasts for a longer period of time (functional hyperplasia, e.g., at body sites that suffer a strong wear and tear). Under these conditions the G1 chalone mechanism is not disturbed.

2. The automatic response to any kind of severe injury (e.g., wounding, removal of the horny layer, treatment with acetic acid) is not only compensatory growth but also a transient loss of susceptibility to G1 chalone which is followed by a sudden development of a pronounced and long-lasting hyperplasia (hyperplastic transformation). In many aspects hyperplastically transformed epidermis resembles neonatal tissue.

In analogy to the hemopoietic system, it is proposed that the proliferative compartment of epidermis consists of different types of stem cells and that only already maturating stem cells are able to respond to G1 chalone. During the hyperplastic transformation this stem cell pool is depopulated due to either hasty keratinization (e.g., after removal of the horny layer) or dedifferentiation (as is discussed for the mechanism of action of tumor promoters). The result is a transient chalone-insensitive cell proliferation.

The concept of dedifferentiation as the driving force of tumor promotion is supported by investigations of effects of hyperplasiogenic and promoting agents on the epidermal adenosine cyclic 3’:5’-monophosphate (cyclic AMP) system. The observations are not consistent with the assumption that epidermal cell proliferation is triggered by a fall in the epidermal level of cyclic AMP but favor the idea that cyclic AMP is involved in the expression of epidermal function. Provided that this is correct certain effects of different phorbol esters on epidermal cyclic AMP may be directly correlated to the “dedifferentiating” and promoting activity of the agent.

Introduction

The great majority of malignant tumors arise from epithelia. Despite this and the fact that epidermis is probably the tissue most widely used in experimental cancer research, our knowledge of the molecular processes that underlie epithelial cell proliferation and differentiation as well as of the proper control mechanisms is very limited. For obvious reasons growth control mechanisms at present are mainly studied with tissue cultures, mostly of mesenchymal or reticuloendothelial origin. There is, however, reason to believe that the mechanisms working in vitro are (in a quantitative and qualitative sense) different from those in the living animal. This may be especially true for highly organized tissues such as the stratified epithelia, the regulatory mechanisms of which probably depend rather strictly on their morphological integrity. Therefore, we believe that the investigation of the control mechanisms of epithelial cell proliferation in the whole animal (although it may be a “dirty” system for many biochemists) is an urgent demand, which will help lead to a proper understanding of neoplastic diseases.

Epithelia such as epidermis suffer permanent wear and tear. This results in a continuous loss of cells. Since this is, at least under normal conditions, precisely matched by the formation of new cells, there must be a stringent control of the rates of cell proliferation and maturation which allows a perfect feedback between cell loss and cell gain. The control of epidermal cell proliferation and differentiation is brought about by a complex pattern of interacting mechanisms which is still far from being understood in detail. Several hormones such as estrogens and androgens, corticosteroids, and catecholamines are involved. Furthermore, the underlying mesenchymal tissue as well as nutritional factors such as vitamin A may play an important role. How-

1 Presented at the Conference “Early Lesions and the Development of Epithelial Cancer,” October 21 to 23, 1975, Bethesda, Md. This work was supported by the Deutsche Forschungsgemeinschaft.
ever, all of these more or less "exogenous" influences may have only a modulatory effect on tissue-own control mechanisms. According to the theory of Weiss and Kavanau (48), a tissue is a self-regulatory system that controls the steady state between cell loss and cell gain by an interplay of stimulatory and inhibitory factors, which are thought to be produced by the tissue itself. These might be, for example, the cyclic nucleotides and the prostaglandins. Both types of substances are known to be synthesized by epidermis; and there is some evidence that cyclic AMP\(^2\) and prostaglandin E\(_2\) inhibit cell proliferation and promote cell maturation, whereas cyclic GMP and prostaglandin F\(_\alpha\) exert the opposite effects. However, the biological activities of cyclic nucleotides and prostaglandins are not tissue specific; instead, they are thought to be the "2nd messengers" of external or local effectors.

In the last 10 years the concept of tissue-own and tissue-specific inhibitors of epidermal cell proliferation has gained increasing interest and support. These "chalone" (6) are thought to be intercellular carriers of information within a feedback circuit between maturing tissue cells (where they are assumed to be synthesized) and the proliferative population (where they exert their effect) (22, 23). The chalone concept still offers by far the most simple and elegant explanation of tissue homeostasis. Therefore, its proof or disproof should be worth the effort. This can be done only by the demonstration and isolation of chalone-like factors.

**Epidermal Chalones**

Since the chalone concept was originally developed to explain certain observations on wounded epidermis (8) and skin treated with carcinogenic hydrocarbons (22, 23, 25, 26), epidermis may be considered to be the "classical" tissue of chalone research.

Ten years ago it was known that aqueous extracts from skin and epidermis are able to inhibit epidermal mitosis in vitro and in vivo (9, 24). In vitro the inhibitory effect was augmented by adrenalin and cortisol (10). It was probably caused by a factor ("epidermal chalone") which could be inactivated by proteases and heat (7). In 1968 Hondius-Boldingh and Laurence (21) reported on the isolation of the inhibitor which was preliminarily characterized as a glycoprotein with a molecular weight between 2.5 and 4 \(\times\) 10\(^4\) daltons. It was exclusively found in and exerted its effect only on epidermis and closely related keratinizing epithelia (30, 37). However, despite this remarkable success doubts were still raised as to the validity of the whole concept. The main reason for scepticism was the fact that the "epidermal chalone" turned out to be a pure inhibitor of mitosis without any effect on DNA synthesis (1, 42). However, in 1969 (20) it was shown that epidermal or skin extracts did in fact inhibit the incorporation of labeled thymidine into epidermal DNA when injected into mice. The inhibition occurred after a lag phase of several hr (20, 32) and as demonstrated later by autoradiography (Refs. 17 and 37; K. Elgjo, personal communication) is due to a delay of the G\(_1\)-S transition rather than to an inhibition of DNA synthesis itself or to a decrease of pool labeling. Although the inhibitor in question is also not species specific and again entirely restricted to keratinizing epithelia (37), it has been shown not to be identical with the antimitotic chalone isolated previously (14, 31). Thus, we have now 2 epidermal chalones, a G\(_1\) chalone retarding the entry of cells into the S phase\(^3\) and a G\(_2\) chalone preventing the transition from G\(_2\) into M phase. Similar observations have been made in other tissues.

In the past 5 years the epidermal G\(_1\) chalone has been highly purified in our laboratory. The starting material was the same pigskin extract used before for the isolation of epidermal G\(_1\) chalone (21). As a test of its inhibitory activity, it was dissolved in 0.9% NaCl solution and injected i.p. into female NMRI albino mice (7 to 8 weeks old). From 12 to 15 hr later, when the inhibitory effect has reached its maximum (32), 35 \(\mu\)Ci of [methyl\(^3\)H]thymidine were injected i.p. After 45 min the animals were killed. Five min prior to sacrifice, the hair of the back was removed by means of a cosmetic depilator (Pilca Creme; Olivan, Wiesbaden, Germany). Immediately after the mice were killed, the depilated skin was dissected and immersed in ice-cold 0.8 M perchloric acid. The epidermis was scraped off with a scalpel (it could also be separated from the dermis by the acetic acid procedure). After homogenization the specific radioactivity of the DNA was measured (32). Most of the G\(_1\) chalone activity was found in the 55 to 72% ethanol precipitate of aqueous pigskin extract. It could be destroyed neither by heating the neutral solution to 100° (for 1 hr) nor by denaturing agents such as phenol, urea, 1 M formic acid (70°), or ionic detergents (100°). It was furthermore completely resistant to trypsin, Pronase, collagenase, RNase, DNase, \(\beta\)-neuraminidase, and hyaluronidase digestion, whereas complete inactivation was achieved with either 0.1 M sodium hydroxide or 1 M hydrochloric acid at room temperature (6 hr). In pigskin extract the factor occurs in a highly aggregated state simulating a molecular weight of several hundred thousand daltons. Due to this complication, the starting material had to be disaggregated prior to any attempt at purification. This was achieved by treatment with dithiothreitol and sodium dodecyl sulfate and afterwards by blocking free sulfhydryl groups by alkylation with iodoacetamide. The material was then fractionated by Pronase digestion, ultrafiltration, precipitation with cetyl pyridinium chloride, and phenol extraction. By this procedure, which has been recently described in more detail (33), an approximately 800,000-fold enrichment of the G\(_1\) chalone over the crude lyophilized skin extract was achieved. A dose level as small as 0.05 to 0.1 \(\mu\)g of the purified factor was sufficient to depress DNA labeling in mouse epidermis in vivo to 50% and more (33). This corresponds to 2 to 4 \(\mu\)g/kg body weight.

Such a high biological activity (which is in the range of that of a hormone), together with tissue specificity and the

\(^{3}\) That epidermal G\(_1\) chalone actually inhibits cell proliferation (and not pool labeling, etc.) has been unequivocally demonstrated by the observation that the depression of thymidine incorporation into epidermal DNA seen after injection of purified G\(_1\) chalone is followed by a corresponding depression of mitotic activity (see Footnote 5).
observation that embryonic, neonatal, and hyperplastic epidermis does not respond to the inhibitor in vivo and in vitro (see below) rule out the possibility that the observed inhibition was the result of unspecific cytotoxic effects. During the purification procedure, almost the whole inhibitory activity of the crude extract was retained, indicating that there is only 1 inhibitor of DNA synthesis in skin. Inhibitory effects measured with crude skin or epidermis extracts may, therefore, be attributed with a certain degree of confidence to the epidermal $G_1$ chalone. However, this holds true only when the effect is measured in vivo and with rather low dose levels, whereas under in vitro conditions there is always the danger of being misled by cytotoxic side effects or the dilution of the labeled precursor pool by unlabeled thymidine and its derivatives, etc.

The purified chalone from pigskin has some peculiar properties. Although, according to a preliminary analysis, it is a (glyco-)peptide which can be extracted with phenol, it is completely resistant to proteolytic digestion. This might be due to the protective effect of carbohydrate residues. On polyacrylamide gels it cannot be made visible with the usual staining methods; most probably it is eluted during the staining procedure, perhaps again due to its high content of carbohydrate (18, 33). Finally, it shows heterogeneity with respect to the molecular size with apparent molecular weights ranging from less than $10^4$ daltons to more than $10^5$ daltons (ultrafiltration, gel chromatography), even after extensive proteolytic digestion and in the presence of detergents. This may indicate some kind of oligomerization and homeopolar aggregation, probably via polysaccharide bridges. In extracts from mouse epidermis most of the chalone activity was found in a fraction between $10^4$ and $10^5$ daltons (J. Schweizer and F. Marks, unpublished results). Therefore, the molecular weight of the "monomer" may be in this range.

According to the feedback hypothesis, the chalone is expected to be synthesized by keratinizing epidermal cells. This is indicated by several lines of evidence: (a) the factor is found exclusively in keratinizing epithelia (37); (b) when keratinizing and basal cells were separated and extracted separately, $G_1$ chalone activity was found almost exclusively in the extract from keratinizing cells (16) whereas $G_2$ chalone was restricted to basal cells (15); (c) extracts from epidermis of 9-day-old chick embryos, which is not yet keratinized, lacked any inhibitory activity. However, when the tissue was cultivated in vitro in the presence of serum the appearance of $G_1$ chalone activity could be demonstrated concomitantly with the development of a horny layer (S. Bertsch and F. Marks, unpublished results); (d) extracts from a nonkeratinizing epidermal carcinoma lacked any inhibitory activity.

On the whole, the epidermal $G_1$ inhibitor fulfills all the requirements of a chalone. Like a hormone it displays its effect in minute amounts. It retards the entry of cells into the phase of DNA replication, it is tissue specific (but not species specific), and it is produced in the course of epidermal cell maturation.

---

F. Marks

---

Epidermal Hyperplasia, Tumor Promotion, and Chalone Mechanism

The successful demonstration of an epidermal chalone may be taken as a proof for the existence of a local feedback regulation of epidermal cell proliferation. Provided this mechanism controls the steady state between the rates of cell death and cell renewal, any influence disturbing this equilibrium (such as wounding or treatment with mitogens) should have a measurable effect on it. Furthermore, this feedback system is expected to play an important role in the development of preneoplastic and neoplastic diseases.

Local treatment of mouse skin with tumor-promoting phorbol esters such as TPA or PDB induces inflammation and strong epidermal hyperplasia. Shortly after the application of the agent and prior to the development of hyperplasia, epidermis no longer responds to injections of $G_1$ chalone (28). After a time interval that depends on the dose level as well as on the type of the promoter (1 to 3 days), the chalone mechanism is gradually reestablished (28). The lack of response cannot be overcome by raising the chalone dose level or by altering the time interval between chalone injection and pulse labeling of DNA. This indicates that the receptor mechanism for chalone has been transiently switched off. There are many complicated but only 2 simple explanations: (a) the mitogen interacts with the receptor molecule, perhaps during an overall attack on the cell membrane; (b) similar to the hemopoietic system (29), epidermis has a heterogeneous stem cell population consisting of chalone-susceptible and chalone-unsusceptible cells. The mitogen stimulates preferentially the unsusceptible cells or induces dedifferentiation of susceptible to unsusceptible cells.

The 2nd assumption is supported by strong evidence that in the hemopoietic system only "late" (already maturing but still proliferating) types of committed stem cells are controlled by chalones, whereas the pluripotential stem cells and the stem cells in the early stages of differentiation do not respond to the inhibitor (L. G. Lajtha, personal communication).

In order to decide between the 2 possibilities mentioned above, epidermal cell proliferation was induced by pure mechanical means. For this purpose the horny layer of depilated mouse back skin was removed by gentle rubbing with cosmetic sandpaper (3), a method that resembles the dermatologist's technique of "stripping" with adhesive tape. After 15 hr, this treatment results in a sharp peak of DNA labeling (3). This is followed by a mitotic wave and, subsequently, by the development of a pronounced epidermal hyperplasia which lasts for at least 5 days. At 9 hr after stimulation, the treated epidermis no longer responded to injections of $G_1$ chalone; only after 48 hr was the responsiveness reestablished. Again the lack of response could not be overcome by increasing the chalone dose level or by altering the time interval between chalone injection and labeling.

---

5 Bertsch, K. Csontos, J. Schweizer, and F. Marks. Effect of Mechanical Stimulation on Cell Proliferation in Mouse Epidermis and Growth Regulation by Endogenous Factors (Chalones). Cell Tissue Kinet., in press.

---

* S. Bertsch and F. Marks. manuscript in preparation.
DNA labeling. These results clearly demonstrate that the loss of chalone responsiveness during the hyperplastic reaction is not necessarily due to an interaction of a chemical mitogen with a receptor site. Furthermore, it is known that the primary response of epidermis to the removal of the horny layer is a hasty keratinization of cells; this occurs prior to the onset of DNA synthesis and leads to a depopulation of the basal cell layer due to an increased vertical cell migration. Those cells are certainly the late stem cells, which under normal conditions are still committed to proliferation and under chalone control. Due to the stripping, the pool of these cells runs empty so that the regeneration of the tissue must now transiently be taken over by "early" stem cells that do not respond to the inhibitor (Chart 1). This course of events is quite similar to that assumed to take place in the hemopoietic system, for example, after severe bleeding.

The cell proliferation induced by stripping still seems to be responsive to the epidermal G2 chalone (3). Thus, a second feedback circuit, perhaps between late and early committed stem cells, may exist (Chart 2). This assumption would offer an explanation for the abundance of G2 chalones in all tissues thus far examined as well as for the localization of epidermal G2 chalone in the basal cell layer (15).

It may be supposed that it is the "breakdown" of the G1 chalone control that ultimately leads to hyperplasia. This is supported when the stimulation of epidermal cell proliferation without subsequent hyperplasia is investigated. Such stimulation can be achieved, for example, by skin massage or by chemical depilation. In both cases the mitotic rate rises nearly 20-fold, and after skin massage the time course and extent of the proliferative events (DNA synthesis, mitosis) is quite similar to that seen after stripping. However, the responsiveness of the tissue to G1 chalone is not lost nor is any hyperplasia developed.

Finally, epidermis which from its morphological appearance seems to be hyperplastic under normal conditions, such as mouse tail epidermis, is nevertheless under chalone control.

From these results we may draw the conclusion that epidermis responds to an external stimulus in different ways.

1. If the stimulus does not involve severe injury, only the flow of cells through the steady state of tissue homeostasis is accelerated. In this case hyperplasia is not observed or develops only very slowly if the stimulus lasts for a longer period of time (functional hyperplasia, for example, at body sites that suffer a strong wear and tear such as the mouse tail). Under these conditions the chalone mechanism is not suspended.

2. The automatic response to any kind of severe injury is not only compensatory growth but also a transient loss of chalone susceptibility which is followed by a sudden development of hyperplasia. This is certainly not due to a simple overshooting of tissue repair but is the consequence of a true "transformation" (induced hyperplasia) probably including the activation of a more undifferentiated stem cell population.

Are there any relationships between the hyperplastic transformation of epidermis and the process of tumor promotion? First it must be stated that a hyperplastic transformation with concomitant loss of chalone responsiveness is induced also by stimuli that apparently do not have a promoting effect, for example, acetic acid. However, promotion is a highly complex process that depends on many different experimental parameters and is still far from being entirely understood. It is quite possible that the induction of epidermal hyperplasia always has promoting consequences if it is permanently repeated; however, in many cases the promoting effect cannot be seen due, for example, to the wrong timing of the experiment or because the "dormant" tumor cells are killed by the hyperplasigenic treatment before they can be promoted. On the other hand, it has been postulated that tumor promotion is the consequence of a dedifferentiation of epidermal cells, a concept that is especially appealing. In this case one would assume that late (chalone-susceptible) stem cells are transformed into early (chalone-unsusceptible) types. The result would be the same as in the case of an accelerated maturation of late stem cells, namely, a changeover of epidermal cell proliferation from a chalone-susceptible to a chalone-unsusceptible population and, consequently, the development of hyperplasia due to an emptying of the pool of late chalone-regulated stem cells (Chart 1).

As long as we do not have proper biochemical parameters of epidermal cell differentiation it is, of course, difficult to decide between the 2 possibilities. However, observations made with newborn mice may show a way to resolve the problem. Embryonic and neonatal mouse epidermis show striking similarities to adult epidermis that has been...
hyperplastic. These analogies are not restricted to morphological features but concern also regulatory functions. Thus, the DNA synthesis in epidermis of newborn animals in vivo or of embryonic mouse or chick epidermis in vitro cannot be inhibited by epidermal G_1 chalone, but the chalone responsiveness is gradually developed within the 1st weeks postnatum (3, 5). Furthermore, we never succeeded in inducing a wave of cell proliferation and hyperplasia in neonatal mouse epidermis, e.g., by stripping or treatment with phorbol esters or acetic acid (3, 27). From these results one may conclude that neonatal epidermis is in the state of hyperplasia and insensitivity to the action of G_1 chalone which in adult epidermis can be induced only by injury. Although insensitive to G_1 chalone, neonatal epidermis responds to G_2 chalone (27). This again supports the hypothesis illustrated by Chart 2.

In light of the concept developed above, this would mean that everyday tissue regeneration in neonatal epidermis is brought about mainly by stem cells that do not respond to chalone. Perhaps the whole tissue, which is clearly not fully developed, is still in a state of "noncommitment" so that any kind of cell maturation must be forced by exogenous factors such as corticosteroids (41) or epidermal growth factor (4) instead of running more or less automatically as is the case of the mature tissue. Under these conditions the pool of chalone-controlled late stem cells may be very small if it exists at all.

The results obtained with newborn animals clearly indicate that chalones are not absolutely necessary for maintaining growth control in epidermis but that they are a condition sine qua non for the maintenance of the adult state. The breakdown of this state and its transition into a "pseudoeMbryonic" state as induced by promoting agents plays certainly a central role in the process of tumor promotion and in the development of preneoplastic alterations. Since it may be the only way in which the new program of tissue differentiation called "cancer" can be realized. It may be recalled in this connection that, in the adult organism, epidermal metaplasia, which is a change of the commitment of the tissue (for example the formation of interfollicular epidermis from hair roots), is observed only after injury. There is certainly no reason to deny that neoplastic development is a special type of a metaplastic reaction.

Epidermal Hyperplasia, Tumor Promotion, and Cyclic AMP

Catecholamines such as adrenalin or isoproterenol are strong inhibitors of epidermal cell proliferation. Originally, adrenalin was regarded as a cofactor of epidermal G_1 chalone (9), but this could not be confirmed. There is, however, evidence that the antimitotic effect of the chalone depends not only on the new program of tissue differentiation called "cancer" but also on the type of the phorbol ester showing a direct correlation with the promoting activity (TPA > phorbol dibenzoate > phorbol dibenzonoate > 4-O-methyl phorbol dibenzoate). Interestingly, the effect is also seen after treatment of mouse skin with 3,4-benzpyrene (43). Thus, the promoting effect of a phorbol ester seems to be related to its ability (a) to depress the responsiveness of epidermal adenylic cyclase to β-adrenergic agonists and (b) to depress after an initial elevation the basal level of cyclic AMP.

Provided that cyclic AMP is involved in epidermal cell growth control in epidermis but that they are a condition sine qua non for the maintenance of the adult state.
Chart 3. The effect of a single local application of phorbol ester (at zero time) on the level of cyclic AMP (cAMP, hatched area), on DNA synthesis, on synthesis of histidine-rich protein (HRP), and on the effect of injections of epidermal G1 chalone on DNA synthesis (right ordinate) in dorsal mouse epidermis. A, 2 nmoles TPA; B, 20 nmoles TPA; C, approximately 500 nmoles PDB; control, animals that were painted with acetone instead of phorbol ester. The data are adopted from Krieg et al. (28) and Grimm and Marks (19).
differentiation, the observations fit very well with the concept of dedifferentiation as the causative event in tumor promotion. One may assume that the early cyclic AMP peak is an expression of the transformation of cells into a less differentiated state, which is afterwards reflected by the subsequent depression of the cyclic AMP level, whereas the late elevation is correlated to the redifferentiation of the tissue. This assumption is supported by several lines of evidence: (a) both peaks of cyclic AMP obviously coincide with 2 peaks of histone phosphorylation (40). According to current concepts this may be regarded as an expression of gene activation (involving cyclic AMP-dependent protein kinases), i.e. during dedifferentiation and redifferentiation; (b) tumor promotion can be inhibited by manipulations that keep the level of cyclic AMP high, i.e., manipulations that prevent the TPA-induced depression (2); (c) the late cyclic AMP peak coincides with the onset of reactions related to epidermal differentiation such as the synthesis of histidine-rich protein and the reestablishment of chalone responsiveness (28).

The change of hormone responsiveness of the epidermal level of cyclic AMP (from a $\beta$-adrenergic to an $\alpha$-adrenergic receptor mechanism) may also be regarded as a reflection of alterations in the state of tissue differentiation. Observations that epidermal adenylyl cyclase of neonatal rats cannot be properly stimulated $\beta$-adrenergically (46) indicate that the $\beta$-adrenergic mechanism is bound to the adult state and that its breakdown after promoter application is an expression of dedifferentiation. Experiments to prove whether or not the $\alpha$-adrenergic mechanism is related to the neonatal state are now in progress.

The effect on the (membrane-bound) $\beta$ receptor may be alternatively interpreted as resulting from an attack of the promoter on the cell membrane, during which the receptor-lipid-adenylyl cyclase complex is destroyed, perhaps due to the surface activity of the phorbol ester. However, this can explain neither the lack of an effect of 4-O-methyl phorbol didecanoate (which is expected to be surface active) nor the effect of anthraline (19) or benzo(a)pyrene (43), which have been shown to be as powerful in inhibiting the $\beta$-adrenergic mechanism as is TPA. Furthermore, it is difficult to understand the relatively long lag phase of 1 to 2 hr between the application of the promoter and the breakdown of the $\beta$ receptor mechanism.

In summary, there is indeed a body of admittedly indirect evidence supporting the concept of dedifferentiation as the driving force for tumor promotion; however, we must collect considerably more experimental data before we can draw final conclusions.

References

Epidermal Growth Control Mechanisms, Hyperplasia, and Tumor Promotion in the Skin

Friedrich Marks

*Cancer Res* 1976;36:2636-2643.

Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/36/7_Part_2/2636

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.