transport. The electrophysiological results provide evidence that both apical and basolateral membrane conductance are coupled to sodium transport. Since replacement of serosal sodium with potassium causes membrane depolarization (*13*), potassium movement during isosmotic potassium-induced cell swelling is probably conductive. Thus, it is likely that the effects of amiloride on this process and on the electrophysiological properties of the basolateral membrane reflect a single pathway for potassium. Since amiloride also inhibits cell volume regulation, it is likely that the volume-regulatory potassium movements also occur through this pathway.

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References and Notes

1. Tight epithelia (urinary bladder, frog skin, renal distal tubule) have transepithelial resistances greater than 500 ohm-cm^2 and potentials greater than 30 mV; in leaky epithelia (gallbladder, proximal tubule), both values are considerably smaller.
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9. Urinary bladders from toads (*Bufo marinus*) were perfused with Ringer solution (composition in m*M*: NaCl, 109; KCl, 2.5; $NaHCO_3$, 2.4; and $CaCl_2$, 0.9; gassed with room air; *p*H about 8.4) on both sides. Electrodes contacted each chamber and cells were impaled with microelectrodes having tip resistances of 18 to 25 megohms and tip potentials of less than 8 mV (*3, 5, 6, 10*). For a discussion of the validity of microelectrode impalements in toad urinary bladder, see (*6*) and A. L. Finn, C. W. Davis, J. Narvarte, in *Ion Transport in Epithelia*, S. G. Schultz, Ed. (Raven, New York, 1981), p. 61.
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11. From Kirchhoff's current law, the amount of current entering and exiting the cell must be equal. The ratio of the voltage deflections ($\Delta V_{mc}/\Delta V_{cs}$) across the membranes is therefore equal to the ratio of membrane resistances, even though the absolute current is not known.
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15. Urinary bladders from bullfrogs rather than toads were selected for the cell volume study because of their superior optical qualities. The transepithelial electrical characteristics of the two tissues are similar (*2*), and preliminary intracellular electrophysiological measurements also indicate a similarity in cell membrane potentials, the ratio of membrane resistances, and in the effects of amiloride on these parameters: thus, $V_{mc} = 41 \pm 8$ mV; $V_{cs} = 49 \pm 13$; the ratio of apical to basolateral membrane resistance = 2.0 ± 0.8; and transepithelial resistance = 2382 ± 689 ohm-cm^2. After amiloride addition, when transepithelial resistance had increased by 61 ± 19 percent, the resistance ratio had changed by -0.18 ± 0.04 (five observations in three tissues).
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A New Tumor-Promoting Agent, Dihydroteleocidin B, Markedly Enhances Chemically Induced Malignant Cell Transformation

Abstract. *Teleocidin, which was isolated from mycelia of Streptomyces, is a potent tumor promoter in mouse skin. The catalytically hydrogenated compound dihydroteleocidin B markedly enhanced malignant cell transformation induced by 3-methylcholanthrene or ultraviolet radiation. Dihydroteleocidin B was at least 100 times more effective in enhancing transformation than 12-O-tetradecanoyl phorbol-13-acetate, the strongest promoter known until now, whereas both promoters showed equal capacities to induce early membrane effects and DNA synthesis.*

The induction of cancer in humans is thought to follow complex interactions between initiators and factors that influence tumor development (*1, 2*). Among such factors are tumor promoters, compounds that are weakly carcinogenic but which markedly enhance the yield of tumors when applied after a low dose of an initiating carcinogen (*2, 3*). In recent years the biological and biochemical effects of promoting agents have been examined extensively with various cell systems. However, the mechanism by which promoting agents convert initiated cells into visible tumors and their etiological significance are still not known.

We report here that dihydroteleocidin B (DHTB), a catalytically hydrogenated compound of teleocidin, which was isolated from mycelia of Streptomyces (*4, 5*), is unusually potent in enhancing 3-methylcholanthrene (MCA)–induced malignant transformation and in inducing morphological alterations in BALB 3T3 cells. DHTB is at least 100 times stronger in enhancing transformation than 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), the strongest promoting agent known until now. On the other hand, DHTB and TPA inhibited the binding of epidermal growth factor and [^{3}H]phorboldibutyrate to cell surface receptors, increased 2-deoxyglucose uptake, and stimulated DNA synthesis in G_0-arrested cells to the same extent, suggesting that the early induction of biochemical responses is not directly related to the enhancement of cell transformation.

Malignant transformation of A31-1-1 cells was assayed by scoring transformation foci as reported previously with BALB 3T3–A31 subclones (*6, 7*). Cells treated with a low dose of MCA (1 μg/ml) alone had very low transformation frequencies (5×10^{-6}). Subsequent treatment with as little as 0.1 ng of DHTB per milliliter for 2 weeks (beginning the fourth day after the removal of MCA) markedly increased the transformation frequency, which was proportional to the concentration of DHTB (Fig. 1). This treatment was found to be optimal for maximizing the transformation frequencies. At the concentrations used (0.1 to 10 ng/ml), DHTB had no cytotoxic effects on the MCA-treated or untreated cells, whether DHTB was added to the culture immediately after cell seeding, immediately after MCA treatment, or 4 days after MCA treatment. TPA increased transformation frequencies only at high concentrations (10 to 1000 ng/ml), as reported for the enhancement of MCA-induced transformation of 10T1/2 cells (*8, 9*). Thus, DHTB is at least 100 times as effective as TPA in enhancing MCA-induced transformation, as shown by comparing concentrations of promoting agents giving the same transformation in A31-1-1 cells. Dose-response tests with various doses of MCA and 10 ng of DHTB or TPA per milliliter indicated that DHTB permits the use of 100-fold lower initiating doses of MCA to achieve the same transformation frequency. Similar marked enhance-

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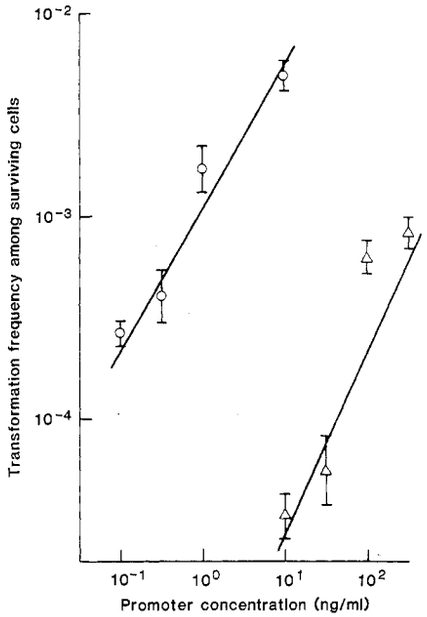

Fig. 1. Transformation frequencies in A31-1-1 mouse cells treated with MCA and subsequently incubated with various concentrations of tumor promoters. Actively growing cells (10^4 cells per 60-mm-diameter plastic dish) were plated in 5 ml of Eagle's minimum essential medium supplemented with 10 percent fetal bovine serum. Twenty-four hours after being plated, the cells were treated with MCA (1 μg/ml) for 72 hours. DHTB (○) or TPA (△) was present in the medium for 2 weeks beginning 4 days after the MCA was removed. The medium was then replaced with fresh, promoter-free medium and the culture was incubated for another 2 weeks, with the medium changed twice a week. The dishes were fixed with methanol and stained with Giemsa 5 weeks after cell plating, and transformed foci were counted. Only foci that showed clear alteration in growth pattern and cell arrangement were counted (*6, 7*). Transformed cells isolated from such foci produce tumors when they are injected subcutaneously into nude mice, whereas nontransformed cells do not (*6, 7*). Each value is the mean ± standard error for eight to ten plates.

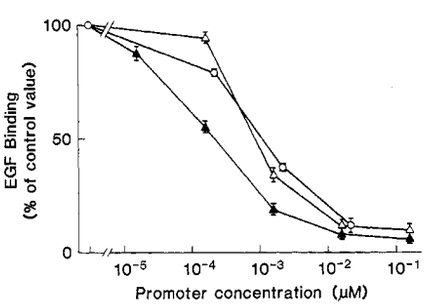

Fig. 2. Inhibition of epidermal growth factor (*EGF*) binding by tumor-promoting agents. A31-1-1 cells (2×10^6) were incubated with 0.2 ng of ^{125}I-labeled EGF (120 mCi/mg; Collaborative Research) in 2 ml of Hanks basic salt solution with various concentrations of DHTB (○), TPA (△), or EGF (▲) for 2 hours and washed five times with Hanks basic salt solution containing 2 mg of ovalbumin per milliliter. Cell-bound radioactivity was counted in a gamma counter after the cells were lysed with 0.1*N* NaOH. The control value was 2200 count/min per dish (100 percent). Values are means ± standard errors for triplicate samples.

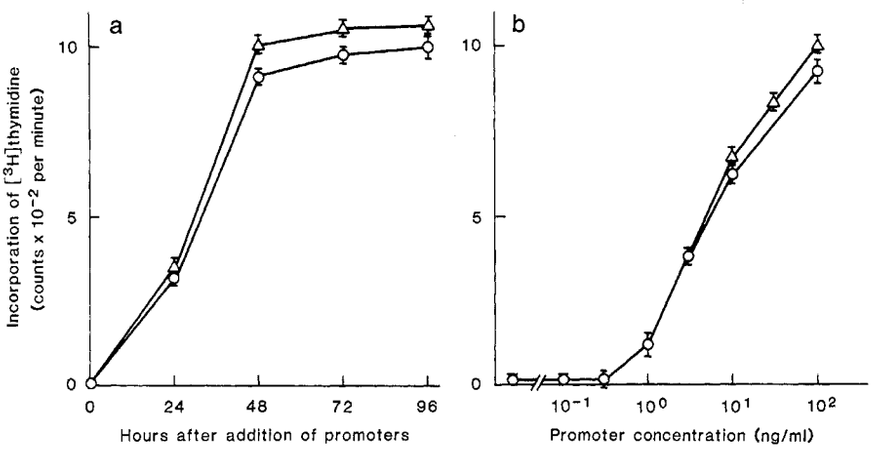

Fig. 3. Induction of DNA synthesis in arrested A31-1-1 mouse cells various times (a) or 48 hours (b) after the addition of promoters at 10 ng/ml (a) or at various concentrations. Cells (3×10^5) were plated in 60-mm-diameter dishes and incubated for 6 days, after which the cells were incubated with depleted medium containing [^{3}H]thymidine (2 μCi/ml) with or without DHTB (○) or TPA (△). To avoid the secondary effects resulting from long exposures to [^{3}H]thymidine, the cells were exposed for 24 hours. Values obtained after 24 hours represent the accumulated uptake of [^{3}H]thymidine derived from separate dishes. At the end of incubation, the radioactive medium was aspirated and the cells were scraped into 2 ml of phosphate-buffered saline, trapped in glass filters, and washed three times with cold 5 percent trichloroacetic acid. The filters were washed twice with cold distilled water, dried, immersed in toluene and diphenyloxazole, and measured for radioactivity in a liquid scintillation counter. Depleted medium was prepared by exposing culture medium containing 10 percent fetal bovine serum for 3 days to confluent cultures of A31-1-1 cells.

ment by DHTB of A31-1-1 cell transformation was also observed when ultraviolet irradiation (*7*) was used as the initiating carcinogen instead of MCA.

In other cell lines, such as Friend leukemia cells (*10, 11*), rat embryo cells (*12*), HL-60 cells (*10*), and human lymphoblastoid cells (*13*), DHTB and TPA have equal potencies in the induction of various biochemical changes that occur within 48 hours after exposure of cells to TPA and which have been suspected to be linked to the tumor-promoting action of these agents (*1, 14–16*). To determine whether there is such a relation, the early biochemical responses induced in A31-1-1 cells by DHTB were compared with those induced by TPA.

One of the early responses of cells to promoting agents is inhibition of the binding of epidermal growth factor to cell surface receptors (*17, 18*). We found that DHTB and TPA (10^{-3} to 10^{-1} μ*M*) inhibited the binding of ^{125}I-labeled epidermal growth factor in A31-1-1 cells equally well (Fig. 2). Another early response to promoting agents is enhancement of the uptake of 2-deoxyglucose (*19, 20*) and certain other nutrients (*1*). DHTB and TPA stimulated equal amounts of 2-deoxyglucose uptake by A31-1-1 cells.

Induction of DNA synthesis in cells arrested at G_0 is a late response to promoting agents and is thought to be the cause of their promoting activity (*21, 22*). The addition of TPA or DHTB to arrested cells induced DNA synthesis at a dose as low as 1 ng/ml; activity was maximal at 100 ng/ml (Fig. 3, a and b). Cell numbers and mitotic rates were also increased equivalently when arrested cells were incubated with DHTB or TPA.

Thus, DHTB and TPA have equal capacities to induce early membrane effects, DNA synthesis, and cell division in A31-1-1 cells arrested at G_0. These results are consistent with the finding that DHTB and teleocidin compete with [^{3}H]phorbol-12,13-dibutyrate for receptor binding to the same extent as TPA. Although TPA induces many biological and biochemical changes in the cells (*3, 21, 22*), the relation between any of the pleiotropic effects of TPA and the enhancement of transformation or tumor promotion is unknown. The different abilities of DHTB and TPA to enhance transformation and their similar abilities to induce certain biochemical changes indicate that some of the biochemical changes induced by promoting agents are not directly related to transformation enhancement or tumor promotion. Indeed, cell variants that are susceptible to the induction of anchorage-independent

growth by TPA do not carry out TPA-induced DNA synthesis at G_0 (*23*).

The effective concentrations of TPA and DHTB for inhibition of epidermal growth factor binding and enhancement of 2-deoxyglucose uptake and the effective concentrations of TPA for enhancement of transformation, stimulation of DNA synthesis in arrested cells, and inhibition of [^{3}H]phorbol-12,13-dibutyrate binding agree with data for other cell lines (*11, 12*), indicating that the susceptibility of A31-1-1 cells to the effects of DHTB and TPA is similar to that of other cell lines. We recently found that DHTB and TPA, applied by painting, enhance the development of skin tumors initiated by 7,12-dimethylbenz[*a*]anthracene equally well (*11*). However, the results shown here suggest that DHTB may be more potent than TPA in promoting tumor development in vivo under certain circumstances.

The extremely low effective dose of DHTB for the enhancement of transformation (less than 10^{-10} g/ml) indicates that environmental promoting agents like teleocidin, the parental compound of DHTB, strongly influence the development of human cancer and that the effects of DHTB reflect interference with the control of cell function by hormone-like substances. Further studies with DHTB and teleocidin are necessary to elucidate these issues.

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The Molecules That Initiate Cardiac Hypertrophy Are Not Species-Specific

Abstract. *Extracts from hypertrophying dog hearts perfused through isolated rat hearts increase the synthesis of messenger RNA and initiate hypertrophy in the treated hearts. Total RNA extracted from experimental and control hearts was translated in vitro and hybridized with polyuridylate. Synthesis of protein and polyadenylate-containing RNA was greater in rat hearts perfused with extracts of hypertrophying dog hearts than in control hearts. The results demonstrate that molecules from hypertrophying dog hearts are not species-specific since they are effective in stimulating transcription of messenger RNA in rat hearts as well as in dog hearts.*

The canine heart, when provoked to hypertrophy by an increased work load, synthesizes water-soluble, extractable molecules that can initiate hypertrophy (as defined by increased protein synthesis) when perfused through isolated, beating (but nonworking) canine hearts (*1*). The possibility that cells generate growth-regulating molecules in response to stress suggests that these molecules may be fundamental to cell function and may exhibit little or no species specificity. If so, extracts derived from the hearts of one species should induce hypertrophy in the hearts of other species. Accordingly, we modified the protocol that we had used for the isolated dog hearts to determine whether canine cardiac extracts would induce responses in rat hearts similar to those in dog hearts.

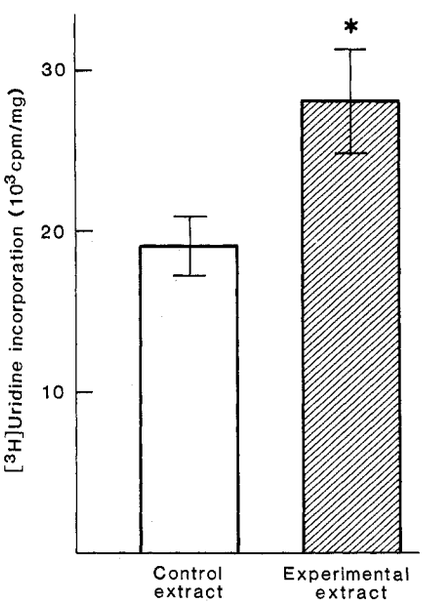

Fig. 1. Incorporation of [^{3}H]uridine into newly synthesized RNA in rat hearts during perfusion. Rat hearts were perfused for 1 hour each with Krebs solution plus normal canine cardiac extract (control extract) and Krebs solution plus hypertrophying canine cardiac extract (experimental extract). Isotope incorporation was 48.7 percent higher in the experimental extract than in the control extract ($t = 2.45$, d.f. = 8, $^*P < .05$). Five hearts were used in each group.

Left ventricular hypertrophy was initiated in dogs by banding the ascending aorta. Six hours later, the heart was extirpated, and the left ventricle was finely chopped. The tissue was frozen with liquid nitrogen and stored at −80°C. Normal canine hearts for control purposes were prepared in identical fashion. On the day of perfusion experiments, 5 to 6 g each of hypertrophying and normal tissue was homogenized in distilled water at a ratio of 1:2 (weight to volume) with a Polytron (Brinkmann) for approximately 15 seconds at the highest speed. The homogenate was centrifuged at 17,000*g* for 30 minutes at 4°C. The supernatant was decanted and placed on ice until used.

Hearts were excised from male, 12-month-old, albino rats. The aortas were cannulated and placed in iced saline. The left ventricle was vented by ventriculot-

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