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Boutwell R K. The function and mechanism of promoters of carcinogenesis. *CRC Crit. Rev. Toxicol.* 2:419-43, 1974.
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The paper reviews data that define the morphological and molecular changes in mouse skin elicited by tumor promoters. Based upon the molecular responses to phorbol esters, the phenotypic changes that accompany carcinogenesis are attributed to gene activations as the processes *sine qua non* of promotion. [The *SCI*® indicates that this paper has been cited in over 640 publications, making it the most-cited paper for this journal.]

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In 1945 Harold Rusch, director of the McArdle Laboratory for Cancer Research at the University of Wisconsin, walked into my laboratory in the Biochemistry Department and asked if, as a member of his staff, I would be interested in applying my experience in nutrition and intermediary metabolism to research on the prevention of cancer. I quickly agreed.

After a few tumor-induction experiments, each six months to a year in duration, M.K. Brush, Rusch, and I found that a relationship exists between the amount of fat in a diet, the caloric intake, and the incidence of chemically induced skin tumors in mice.[1] As a first step toward explaining the phenomenon in molecular/metabolic terms, we reported that a shift in the balance of trophic hormones secreted by the pituitary accompanied caloric restriction.[2] As a part of this change in hormone balance, the increased cortisone production caused specific metabolic changes.

We also learned, in attempting to shorten the time required for carcinogenesis, that higher doses of carcinogen abolished the protective effects of certain diets. Hence, I was intrigued by J.C. Mottram's report[3] that a single application of a carcinogen to mouse skin, at a dose so low that no visible changes were detectable, caused a permanent but cryptic change at the site. Subsequent repeated applications of croton oil to the same site elicited many benign tumors within 12 weeks; yet an identical regimen of croton oil to skin that had not been pretreated did not cause any tumors in 12 weeks.

The processes resulting from this sequential treatment were named initiation and promotion, respectively. Using Mottram's model system, we found that the promotion component was inhibited almost completely by caloric restriction and by adrenal steroids. Thus began a long-term program to learn the metabolic effects of promoting agents on mouse skin, with the ultimate goal of establishing rational means to prevent neoplasia.

My first papers on the relationship between the biochemical responses in mouse skin and the promotion of skin tumors were published 30 years ago. They aroused little interest beyond that of perhaps a dozen people who were investigating the biology of tumor promotion. However, after the active component of croton oil (12-O-tetradecanoylphorbol-13-acetate, known as TPA or PMA) became available, and, due to the efforts of outstanding graduate students and postdoctoral fellows in my laboratory during that period (W.M. Baird, G.T. Bowden, N.H. Colburn, C.T. Helmes, H. Hennings, T.G. O'Brien, P.W. Melera, C.C. Muckerman, J.D. Scribner, T.J. Slaga, R. Raineri, and L.R. Rohrschneider), progress toward understanding the molecular changes that are an essential part of the process of promotion in skin moved forward rapidly. It was possible to write a comprehensive review (integrating data from a number of their publications) that demonstrated that gene activation is the hallmark of tumor promotion. This review brought together for the first time the supporting data for that conclusion.

Initially, the review received at best a mixed reception, probably because it stood alone. However, after three or four years it was widely recognized that both cultured cells and *in vivo* systems show a pleiotypic response to nanomolar quantities of specific phorbol esters. For example, it was found that TPA induces increased activity of the polyamine biosynthetic enzyme ornithine decarboxylase of up to several hundredfold both *in vivo*[4] and *in vitro*. A second example, the association of protein kinase C activity with the TPA receptor, emanated from several sources including groups associated with Y. Nishizuka,[5] J.E. Niedel,[6] and C.L. Ashendel[7] among others. Knowledge based on the pleiotypic responses to TPA has contributed to the recognition of the variety of processes that may be involved in chemical carcinogenesis by diverse agents. Furthermore, model systems have been devised in which TPA causes the expression of oncogenes.[8] Data obtained with TPA, as a consequence of its gene-activating properties, suggest convergence in the processes that are involved in carcinogenesis regardless of causative agents. [In 1979 Boutwell received the Clowes Award, administered by the American Association for Cancer Research and supported by Eli Lilly and Company; this work contributed to the award of this honor.]

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4. **O'Brien T G, Simsiman R C & Boutwell R K.** Induction of the polyamine biosynthetic enzymes in mouse epidermis by tumor promoting agents. *Cancer Res.* 35:1662-70, 1975.
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8. **Brown K, Quintanilla M, Ramsden M, Kerr I B, Young S & Balmain A.** V-ras genes from Harvey and BALB murine sarcoma viruses can act as initiators of two-stage mouse skin carcinogenesis. *Cell* 46:447-56, 1986.