4 E. DeRobertis, G.S. Lunt and J.L. LaTorre, Molec. Pharmac. *7*, 97 (1971).
5 J. Folch-Pi and P.J. Stoffyn, Ann. N.Y. Acad. Sci. *195*, 86 (1972).
6 E.F. Soto, J.M. Pasquini, R. Plácido and J.L. LaTorre, J. Chromat. *41*, 400 (1969).
7 S.R. Levinson and R.D. Keynes, Biochim. biophys. Acta *288*, 241 (1972).
8 S. Udenfried, S. Stein, P. Böhlen, W. Dairman, W. Leimgruker and M. Weigele, Science *178*, 871 (1972).
9 E. Heilbronn, in: Cholinesterases and Cholinergic Receptors, p. 395. Ed. E. Reiner. Croatica Chemica Acta, Zagreb, 1975.

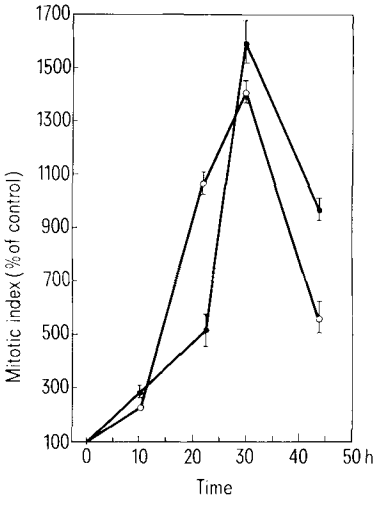
Induction of epidermal cell proliferation by a tumour promoter in vitamin B_6-deficient mice

A.W. Murray[1,2]

School of Biological Sciences, Flinders University of South Australia, Bedford Park (South Australia, 5042), 24 October 1977

Summary. Topical application of the tumour promoter 12-0-tetradecanoyl-phorbol-13-acetate to skin caused a marked enhancement of mitotic activity both in mice maintained on a complete diet or on a vitamin B_6-deficient diet.

The enzyme ornithine decarboxylase catalyses the rate-limiting step in the biosynthesis of the polyamines in mammalian tissues[3]. The activity of the enzyme is enhanced by a wide range of physiological stimuli in a number of tissues. These stimuli are frequently associated with an increased rate of cellular proliferation, leading to the fairly general conclusion that induction of the decarboxylase is of significance in growth regulation[4-7]. The tumour promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA) causes an early rise in mouse epidermal ornithine decarboxylase activity[8], followed by marked hyperplasia[9]. However, enhancement of epidermal cell proliferation by a range of stimuli is not invariably preceded by an increase in decarboxylase activity[8] and the 2 events may not be causally linked, at least in this tissue. Ornithine decarboxylase requires pyridoxal 5'-phosphate as a cofactor[10], and it has recently been shown that maintenance of mice on a vitamin B_6-deficient diet for 14 days greatly decreases the induction of decarboxylase in epidermis by TPA[11]. The purpose of the present study was to determine whether this decreased response modified the ability of TPA to enhance the proliferative activity of epidermal cells.



Mitotic indices were determined in epidermis at varying times after treatment with TPA. Animals were maintained on a complete diet (○) or a vitamin B_6-deficient diet (●) during the experiment and for 14 days prior to TPA treatment. Each point represents the mean ± SEM of results obtained with 3 (10-h point) or 4 (other time points) separate animals.

Materials and methods. Female Swiss albino mice were maintained for 14 days on either a complete or vitamin B_6-deficient diets as described before[11]. Animals were treated with TPA (17 nmoles in 0.2 ml acetone) or with acetone (0.2 ml). At varying times after treatment, groups of animals were injected i.p. with 0.1 mg colchicine (in 0.2 ml 0.9% NaCl) and were sacrificed after a further 2 h. Samples of skin were taken for histology as described before[12]; the mitotic index is expressed as the number of metaphase cells per 100 nucleated, interfollicular cells.

Results and discussion. It is clear from the results shown in the figure that TPA induced a marked proliferative response in the epidermis of mice maintained on both the complete and vitamin B_6-deficient diet. There was some suggestion of a more rapid early rise in mitotic activity in the control group, but in general the shape of the response curves was similar. The initial basal mitotic index in the B_6-deficient animals was 0.54 ± 0.06 and in the control animals 0.78 ± 0.09. These values were not significantly different at the 5% level. Mitotic indices determined in acetone-treated control animals (maintained on either a complete or B_6-deficient diet) at 22, 30 or 44 h did not differ from zero time control animals.

It was previously reported that the induction of epidermal ornithine decarboxylase by TPA in mice maintained on the deficient diet for 14 days was only about 5% of that in mice kept on a complete diet[11]. Consequently, the present data do not support a causal relationship between ornithine decarboxylase and induction of proliferative activity in mouse epidermis.

1 Acknowledgment. The author thanks Mr P. Daenke for excellent technical assistance.
2 This investigation was supported by the Australian Research Grants Committee and the Australian Tobacco Research Foundation.
3 C.W. Tabor and H. Tabor, A. Rev. Biochem. *45*, 285 (1976).
4 C.V. Byus, G.A. Hedge and D.H. Russell, Biochim. biophys. Acta *498*, 39 (1977).
5 Z.N. Canellakis and T.C. Theoharides, J. biol. Chem. *251*, 4436 (1976).
6 E.W. Gerner and D.H. Russell, Cancer Res. *37*, 482 (1977).
7 P.S. Mamont, P. Bohlen, P.P. McCann, P. Bey, F. Schuber and C. Tardif, Proc. natl Acad. Sci. *73*, 1626 (1976).
8 T.G. O'Brien, Cancer Res. *36*, 2644 (1976).
9 F. Marks, Cancer Res. *36*, 2636 (1976).
10 A.E. Pegg and H.G. Williams-Ashman, Biochem. J. *108*, 533 (1968).
11 A.W. Murray and M. Froscio, Biochem. biophys. Res. Commun. *77*, 693 (1977).
12 A.K. Verma and A.W. Murray, Cancer Res. *34*, 3408 (1974).