Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 1062–1066, February 1981
Cell Biology

Alterations in polyamine levels induced by phorbol diesters and other agents that promote differentiation in human promyelocytic leukemia cells

(tumor promoters/putrescine/retinoic acid/anthralin/ornithine decarboxylase)

ELIEZER HUBERMAN, CHARLES WEEKS*, ADRIA HERRMANN, MICHAEL CALLAHAM, AND THOMAS SLAGA

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Communicated by Gerald N. Wogan, November 10, 1980

ABSTRACT **Polyamine levels were evaluated in human HL-60 promyelocytic leukemia cells after treatment with inducers of terminal differentiation. Differentiation in these cells was determined by increases in the percentage of morphologically mature cells and in lysozyme activity. Treatment of the HL-60 cells with phorbol 12-myristate-13-acetate (PMA), phorbol 12,13-didecanoate or other inducers of terminal differentiation such as dimethylsulfoxide and retinoic acid resulted in increased levels of putrescine. However, no increase in putrescine could be detected after PMA treatment of a HL-60 cell variant that exhibited a decreased susceptibility to PMA-induced terminal differentiation. Similarly, no increase in putrescine was observed with two non-tumor-promoters (phorbol 12,13-diacetate and 4-*O*-methyl-PMA) or with anthralin, a non-phorbol tumor promoter. In addition to enhancing putrescine levels, PMA also increased the amount of spermidine and decreased the amount of spermine. The increase in putrescine and spermidine preceded the expression of the various differentiation markers. Unlike the changes observed in the polyamine levels after PMA treatment, the activities of ornithine and S-adenosylmethionine decarboxylases, which are polyamine biosynthetic enzymes, did not significantly change. α-Methylornithine and α-difluoromethylornithine and methylglyoxal bis(guanylhydrazone), which are inhibitors of the polyamine biosynthetic enzymes, did not affect differentiation in control or PMA-treated cells. Because of these observations, we suggest that the change in polyamine levels involve biochemical pathways other than the known biosynthetic ones. By-products of these pathways may perhaps be the controlling factors involved in the induction of terminal differentiation in the HL-60 and other cell types as well.**

Phorbol 12-myristate-13-acetate (PMA) and related phorbol diesters, which are plant diterpenes (1), promote the formation of skin tumors after initiation by a low dose of a carcinogen (2, 3). In some cultured cell types, these phorbol esters inhibit spontaneous or induced terminal differentiation (4–11). In other cells (12–16), including the human HL-60 promyelocytic leukemia cells (17–20), rather than inhibiting, they can induce such a change. Differentiation in the HL-60 cell line can be characterized by inhibition of cell growth, attachment of cells to the surface of Petri dishes (17–19, 21), changes in cell structure (17, 19, 20), increase in percentage of phagocytizing cells (17), and stimulation of acid phosphatase and lysozyme activities (19, 20, 22). In addition to inducing these phenotypic changes, PMA induces the expression of quiescent ribosomal RNA genes in a human–mouse hybrid cell line (23). Thus, the PMA-induced terminal differentiation may result from alterations in the expression of genes associated with the differentiated state.

The physiologically occurring polyamines putrescine, spermidine, and spermine (24, 25) (Fig. 1) have been shown to be involved in the regulation of cell growth (24–27) and in tumor promotion (28). It was also recently suggested that they may play a role in the control of cell differentiation (29–32; †). The present studies therefore were undertaken to determine the possible involvement of polyamines in the control of cell growth and differentiation in human HL-60 promyelocytic leukemia cells that can be induced to differentiate by various agents including the tumor-promoting phorbol diesters.

MATERIALS AND METHODS

Chemicals. PMA, phorbol 12,13-diacetate (PDA), phorbol 12,13-didecanoate (PDD), and 4-*O*-methylphorbol 12-myristate-13-acetate (4-*O*-MePMA) were obtained from Peter Borchert (University of Minnesota, Minneapolis, MN). The phorbol esters were dissolved in a final concentration of 0.1% dimethyl sulfoxide; dimethyl sulfoxide at this concentration in culture medium did not affect cell growth, phagocytosis, lysozyme activity, morphological differentiation, or polyamine levels. Putrescine, spermidine, spermine, and dimethyl sulfoxide were obtained from Sigma, α-methylornithine was from Calbiochem, and methylglyoxal bis(guanylhydrazone) (MGBG) was from Aldrich. α-Difluoromethylornithine (F_2MeOrn) was a gift from the Centre de Recherche Merrell International (Strasbourg, France). DL-[1-^{14}C]Ornithine (50 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), and *S*-[*carboxyl*-^{14}C]adenosyl-L-methionine (55 mCi/mmol) were purchased from New England Nuclear.

Cells and Culture Conditions. The human promyelocytic HL-60 leukemia cells (33) were provided by R. C. Gallo (National Cancer Institute, Bethesda, MD). The cells were cultured in bacterial plastic Petri dishes (no. 1007, Falcon) in RPMI-1640 medium plus 20% fetal calf serum supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) (GIBCO). For the polyamine and lysozyme determination, materials pooled from 5–10 100-mm Petri dishes were used. Each Petri dish was inoculated with 2×10^6 cells in logarithmic growth in 9 ml of medium and was treated with the phorbol esters or other compounds 24 hr later.

Evaluation of Morphological Differentiation. For morphological assessment of cell differentiation, control and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PMA, phorbol 12-myristate-13-acetate (12-*O*-tetradecanoylphorbol 13-acetate); PDA, phorbol 12,13-diacetate; PDD, phorbol 12,13-didecanoate; 4-*O*-MePMA, 4-*O*-methylPMA; ODCase, ornithine decarboxylase; AdoMetDCase, S-adenosylmethionine decarboxylase; F_2MeOrn, α-difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone).

* To whom reprint requests should be addressed.

† Weeks, C. E., Slaga, T. J. & Huberman, E., Third International Conference on Differentiation: Differentiation and Neoplasia, Minneapolis, MN, Aug. 28–Sept. 1, 1978, p. 45 (abstr.).

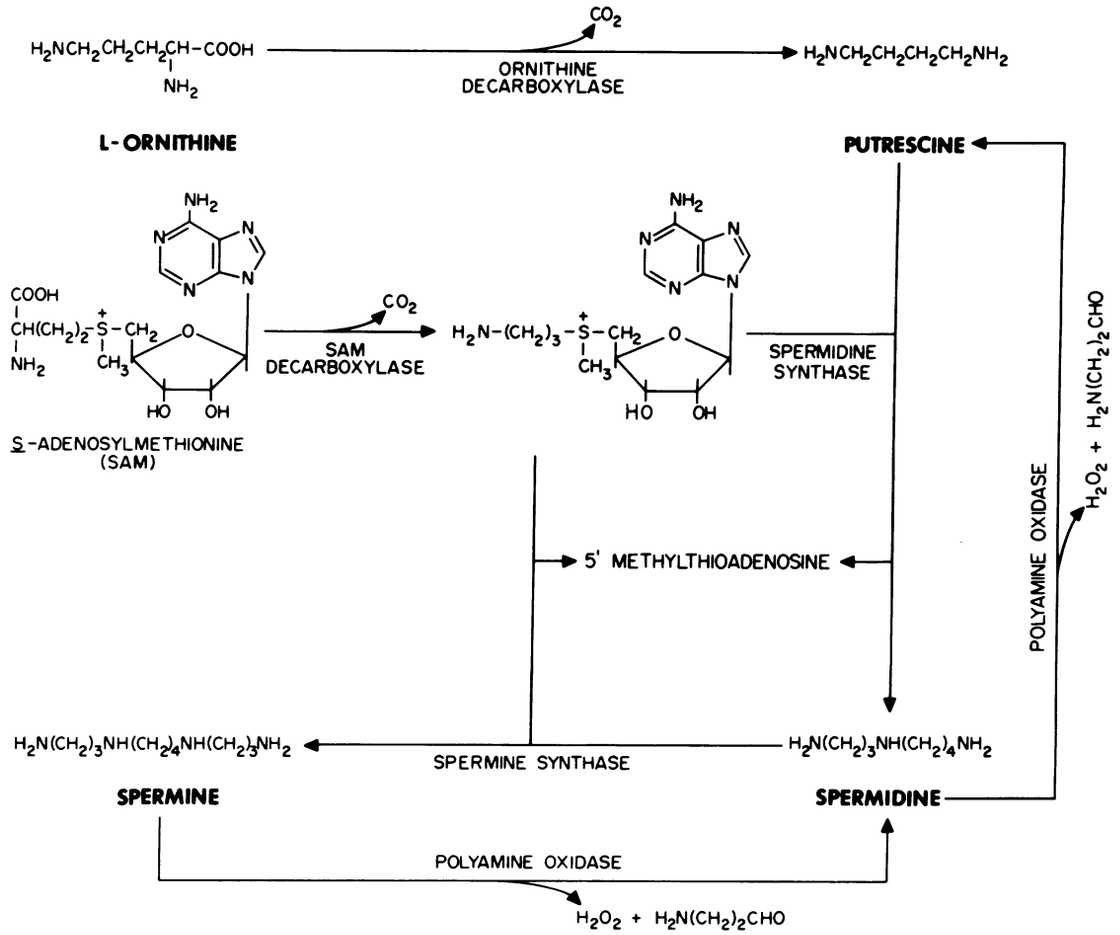

FIG. 1. Scheme of mammalian polyamine biosynthetic pathways.

treated cells were removed from the Petri dishes, centrifuged, and resuspended in 1 ml of growth medium. Aliquots (≈0.1 ml) of the cell suspension were spread on a glass slide, air dried, and stained with Wright/Giemsa. Differential cell counting was performed on 200–400 stained cells from at least two preparations for each experimental point.

Determination of Lysozyme Activity. Control and treated cells were harvested and washed twice with phosphate-buffered saline for determination of lysozyme activity (34). The cell pellets were lysed for approximately 10 min at 37°C in 1 ml of 67 mM potassium phosphate, pH 6.24/0.2% Triton X-100. Cell debris was removed by centrifugation at 500 × *g* for 5 min. Lysozyme activity was determined by a decrease in turbidity of a cell suspension of *Micrococcus lysodeikticus* (OD at 450 nm) at 25°C, as described in Sigma Bulletin L-6876. The reaction mixture (3 ml) contained 1.3 ml of substrate (0.3 mg of bacteria per ml in phosphate-buffered saline), 0.2 ml of cell lysate, and 1.5 ml of 67 mM potassium phosphate, pH 6.24/0.2% Triton X-100. The growth medium from which the cells had been harvested was also assayed for lysozyme activity. To determine this activity, we used 1.3 ml of substrate, 0.5 ml of growth medium, and 1.2 ml of buffer. The μg equivalent of lysozyme activity was estimated by comparing its activity to that of a standard activity curve for purified hen egg white lysozyme (Sigma product L-6876). Results are expressed as total amount of lysozyme in μg equivalents per 10^7 cells per 10 ml of medium. The activity of lysozyme released by the HL-60 cells into the culture medium did not change upon incubation at 37°C for 5 days in a humidified incubator.

Polyamine Analysis and Ornithine and *S*-Adenosylmethionine Decarboxylase Assays. Ornithine decarboxylase (ODCase) and *S*-adenosylmethionine decarboxylase (AdoMetDCase) were determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine and *S*-[*carboxyl*-^{14}C]adenosyl-L-methionine, respectively (35, 36). Polyamines were quantitated by high-pressure liquid chromatography of dansylated derivatives as described (35). Cells were harvested and washed three times in cold phosphate-buffered saline and then lysed by two freezing/thawing in dry ice/acetone in 0.5 or 1.0 ml of 50 mM sodium/potassium phosphate, pH 7.2/5mM dithiothreitol/0.1 mM EDTA/0.2 mM pyridoxal 5'-phosphate for ODCase activity assessment or lysed in 0.3 M $HClO_4$ for polyamine analysis (35). Putrescine-stimulated AdoMetDCase activity was measured by modification of reported methods (36). Putrescine was added, in 1.0 ml final volume, at 2.5 mM and *S*-adenosylmethionine at 0.2 mM (0.4 μCi of [^{14}C]adenosylmethionine). Aliquots (0.1–0.2 ml) of the supernatant obtained after centrifugation at 20,000 × *g* for 30 min were used for the assays. ODCase and AdoMetDCase activities are expressed as nmol of CO_2 per mg of protein per hr and per 30 min, respectively, and polyamine values are nmol of polyamine per mg of DNA (determined by the diphenylamine reaction).

RESULTS

Cell Differentiation and Alteration in Polyamine Levels Induced by PMA. During a 24-hr period of growth in suspension, untreated HL-60 promyelocytic leukemia cells contained about

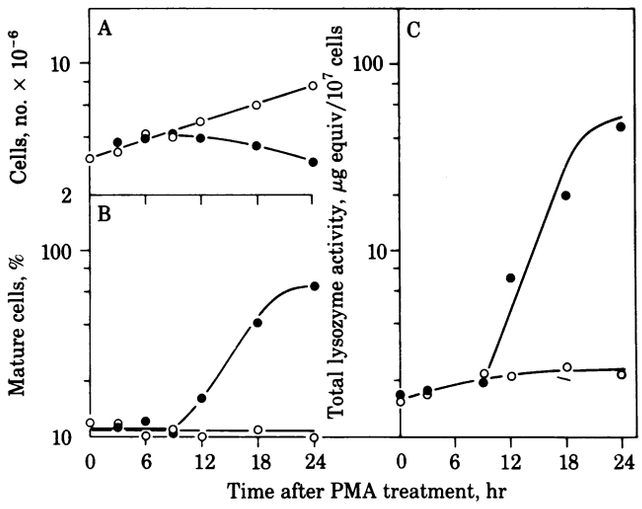

FIG. 2. Cell growth (*A*), proportion of morphologically mature cells (*B*), and lysozyme activity (*C*) in HL-60 cells at different times after treatment with 0.5 nM PMA. The cultures were treated 1 day after 2 × 10^6 cells had been seeded in 10 ml of growth medium in 100-mm Petri dishes. The morphologically mature cells were composed mainly of myelocyte- and metamyelocyte-like cells and a small fraction of banded and segmented neutrophil-like cells. ●, PMA; ○, control.

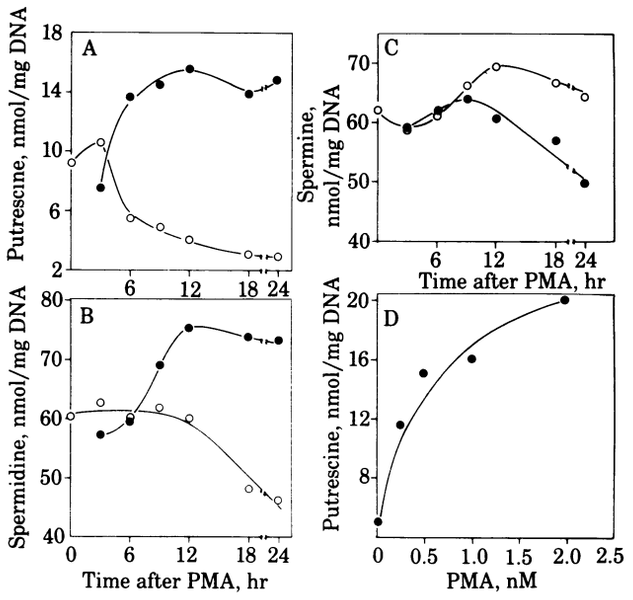

FIG. 3. Level of putrescine (*A*), spermidine (*B*), and spermine (*C*) in HL-60 cells at different times after treatment with 0.5 nM PMA. (*D*) Amount of putrescine at 12 hr after treatment with PMA at different concentrations. The increased level of putrescine and spermidine as well as the decrease in spermine were highly reproducible in six separate experiments. The above results represent data from a typical experiment. ●, PMA, ○, control.

10% morphologically mature cells (myelocytes, metamyelocytes, and banded and segmented cells) and exhibited a total lysozyme activity of about 2 μg of lysozyme equivalents per 10^7 cells (Fig. 2). About 50–75% of this activity was detected in the cells, and the remainder was in the culture medium. During this period, putrescine decreased from about 10 to 3 nmol/mg of DNA; spermidine decreased after 12 hr from about 60 to 50 nmol/mg of DNA (Fig. 3).

After a lag period of 9 hr, PMA at a dose of 0.5 nM, which completely inhibited cell replication, caused the attachment of the HL-60 cells to the surface of the Petri dish and increased the percentage of morphologically mature cells and lysozyme activity. By 24 hr after PMA treatment, the cells consisted of 60% morphologically mature cells and exhibited 50 μg equivalent of lysozyme activity per 10^7 cells (Fig. 2). Of this activity, only a small fraction (0.5–1.5 μg equivalents per 10^7 cells) was detected in the cells. These results indicate that PMA not only increased lysozyme activity in the HL-60 cells but also caused its release to the medium.

In addition to these changes, PMA caused an increase in the levels of putrescine and spermidine. After 3 hr of treatment with 0.5 nM PMA, the amount of putrescine increased from about 8 to 15 nmol/mg of DNA, reaching a plateau after 9 hr of treatment (Fig. 3*A*). The increase in putrescine levels was dose dependent and by 12 hr after treatment with 2 nM PMA, putrescine was increased to 20 nmol/mg of DNA (Fig. 3*D*). Spermidine levels were also enhanced from about 60 to 75 nmol/mg of DNA by 12 hr, after which no further increase was observed. Unlike putrescine and spermidine, no increase in spermine levels could be detected after PMA treatment. On the contrary, in the period between 9 and 24 hr after PMA treatment, spermine decreased from 65 to 50 nmol/mg of DNA (Fig. 3*C*). During these experiments, no significant changes in the polyamine levels could be detected in the growth medium.

These results indicate that PMA treatment caused an increase in putrescine and spermidine in the HL-60 cells and this increase could be detected prior to the expression of the differentiation markers such as cell attachment, morphological maturation, and increase in lysozyme activity.

Increase in Putrescine Levels by Other Inducers of Terminal Differentiation. Putrescine levels in the HL-60 cells were also determined after treatment with PDD, another tumor-promoting phorbol diester, and with two non-tumor-promoting phorbol diesters, PDA and 4-*O*-MePMA. In addition, we included in our analysis a non-phorbol diester tumor promoter, anthralin (37) as well as dimethyl sulfoxide and retinoic acid, which are not generally considered to be tumor promoters although the latter has been found to enhance UV-induced skin carcinogenesis (38). Dimethyl sulfoxide and retinoic acid (33, 39), unlike anthralin, can induce terminal differentiation in the HL-60 cells. Anthralin at 4 mM inhibited the growth of HL-60 cells but did not produce an increase in the percentage of morphologically mature cells; neither did it increase lysozyme activity, indicating that inhibition of cell growth is not sufficient to induce differentiation in the HL-60 cells. PMA, PDD, dimethyl sulfoxide, and retinoic acid, all of which can induce terminal differentiation in HL-60 cells, yielded putrescine levels that were higher than control (Table 1). PDA, 4-*O*-MePMA and anthralin, which are not inducers of differentiation in these cells, did not exhibit such an effect. These results suggest that a relationship can be established between the ability of chemicals, including phorbol diesters, to induce terminal differentiation in the HL-60 cells and their ability to cause an increase in cellular levels of putrescine.

Polyamine Levels in HL-60 Cells with a Decreased Susceptibility to the Induction of Differentiation by PMA. A cell variant, designated R-20, resistant to the growth inhibition effect of PMA was isolated by culturing the HL-60 for 20 subcultures (5- to 8-day intervals) in the presence of 0.5 nM PMA. These variant cells exhibit a decreased susceptibility to PMA induction of morphological maturation and enhancement of lysozyme activity. Likewise, these cells did not show an increase in putrescine levels after PMA treatment (Table 2). However, like the parent HL-60 cells, these cells could be induced to differentiate by other inducers such as retinoic acid and dimethyl sulfoxide.

Table 1. Relationship between morphologically mature HL-60 cells and putrescine level after various treatments

Treatment*	Tumor-promoting activity	Morphologically mature cells, %†	Putrescine, nmol/mg DNA‡
Anthralin	+	11	3
PDA	−	12	3
4-*O*-MePMA	−	15	4
PDD	+++	70	10
PMA	+++	85	13
Me_2SO	−	80	8
Retinoic acid	−	85	9

* The HL-60 cells were treated with phorbol esters at 0.5 nM, dimethyl sulfoxide (Me_2SO) at 0.2 M, retinoic acid at 1 μM, and anthralin at 0.4 mM.

† The percentage of the morphologically mature cells was determined 2 days after incubation with phorbol diesters and 5 days after incubation with dimethyl sulfoxide, retinoic acid, or anthralin. The control contained 12% and 15% mature cells after 2 and 5 days, respectively. In contrast to the phorbol diesters, dimethyl sulfoxide and retinoic acid gave a higher fraction of cells with banded and segmented nuclei.

‡ Putrescine levels were determined 12 hr after treatment with the inducer. The control value was 4 nmol/mg of cellular DNA. The differences in putrescine values between treated and control were highly reproducible in four separate experiments. The above results represent data from a typical experiment.

The relationship between the ability of four phorbol diesters, anthralin, dimethyl sulfoxide, and retinoic acid to act as inducers of terminal differentiation and their ability to stimulate putrescine levels in the HL-60 cells and the absence of increased putrescine levels in the PMA-resistant cells suggests that the alterations in polyamine levels are associated with the induction of cell differentiation in the HL-60 cells.

Polyamine Biosynthesis Enzyme Activities. To determine whether the increase in putrescine levels was due to a stimulation of polyamine biosynthetic enzymes, we tested ODCase and AdoMetDCase activities in both the control and PMA-treated HL-60 cells. During the 1.5–9 hr after treatment with 0.5 nM PMA, the ODCase activities from the control and treated cells were similar, ≈1.5 nmol of CO_2 per mg of protein per hr. By 24 hr, these activities decreased to about 0.5 nmol of CO_2 per mg of protein per hr. These results suggest that the increase in putrescine induced by PMA is not due to a stimulation of ODCase activity. We have also tested F_2MeOrn, an irreversible ODCase inhibitor (40), for its effect on polyamine biosynthesis and cell differentiation in HL-60 cells. F_2MeOrn at 1–5 mM was added 2 hr prior to treatment with PMA and was present during a 12-hr period of the PMA treatment for polyamine analysis and a 48-hr period for cell differentiation assessment. F_2MeOrn decreased putrescine in the control and PMA-treated HL-60 cells to undetectable levels, decreased spermidine to less than 50% of its normal levels, and inhibited cell growth but did not affect control or PMA-induced cell differentiation. As in the case of F_2MeOrn, the reversible ODCase inhibitor α-methylornithine (26, 27, 41) did not affect cell differentiation. This indicates again that a decrease in cell proliferation is not always associated with differentiation in these cells. The effect of PMA on AdoMetDCase activity was negligible over a 24 hr period after treatment. Values in control and PMA treated cultures ranged from 0.3 to 0.6 nmol of CO_2 per mg of protein per 30 min. The AdoMetDCase and diamine oxidase inhibitor MGBG (36), at 1–100 μM did not alter spontaneous or PMA-induced cell differentiation when tested under conditions similar to those described for F_2MeOrn.

DISCUSSION

In the present studies we have shown that chemicals such as the phorbol diesters PMA and PDD, which are potent tumor promoters (1, 17–20), and other inducers of terminal differentiation in the HL-60 cells such as dimethyl sulfoxide (33) and retinoic acid (39) can cause an increase in the cellular level of putrescine. Such an increase in putrescine after treatment with PMA could not be detected in an HL-60 cell variant which exhibited a decreased susceptibility to PMA-induced terminal differentiation. Other chemicals, such as the non-phorbol ester-type tumor promoter anthralin (37) and the non-tumor-promoting phorbol diesters 4-*O*-MePMA and PDA (1), which do not induce terminal differentiation (17–20), were inactive. The enhancement of putrescine levels by PMA preceded the expression of the various differentiation markers such as an increase in morphological mature cells, increase in lysozyme activity, and cell attachment (17–22).

It has been suggested that, in mammalian cells, increased ODCase activity and polyamine levels may be a prime factor in the stimulation of DNA synthesis and cell replication (24, 25, 42), although in some studies a dissociation between ODCase activity and DNA synthesis was observed (43, 44). PMA treatment, which in the HL-60 cells inhibits DNA synthesis (21) and cell growth and induces terminal differentiation (17–20), causes an increase in putrescine and spermidine levels and a decrease in the level of spermine without affecting ODCase and AdoMetDCase activities. These results suggest that the increase in putrescine and spermidine levels may involve biochemical pathways other than the known biosynthetic ones (Fig. 1). Our preliminary studies with labeled putrescine and spermine indicated that the synthesis of polyamines in the HL-60 cells can follow the known route of putrescine to spermine via spermidine as well as the reverse cycle from spermine to spermidine (Fig. 1). However, no significant conversion of spermidine to putrescine could be detected (unpublished data). Thus, the putrescine increase could occur by another, as yet unknown, pathway. The decrease of spermine levels in the PMA-treated cells, which may result from an increase in the conversion of spermine to spermidine with liberation of an aminoaldehyde and H_2O_2 (Fig. 1) (45), perhaps may be a controlling factor in PMA-induced cell differentiation. It therefore would be of interest to study these products, as well as 5′-methylthioadenosine, the by-product of spermidine and spermine

Table 2. Putrescine levels and induction of terminal differentiation in HL-60 cells and in PMA-resistant R-20 cells after treatment with 0.5 nM PMA

	Cells, no. $\times 10^{-6}$		Morphologically mature cells, %		Lysozyme, μg equiv./10^7 cells		Putrescine, nmol/mg DNA	
Treatment	HL-60	R-20	HL-60	R-20	HL-60	R-20	HL-60	R-20
Control	6.3	7.2	12	10	2.3	2.0	4	6
PMA	0.8	5.9	85	25	96	12	16	7

Cell growth, morphological differentiation, and lysozyme activities were determined 2 days after treatment PMA; putrescine was measured after 12 hr of treatment.

biosynthesis (24, 25), for their effect on cell growth and cell differentiation in the HL-60 cells. Another factor influencing the altered polyamine levels observed after PMA treatment may be changes in polyamine acetylation patterns (25).

An interesting possibility is that PMA alters polyamine levels by affecting transglutaminases that can use polyamines as their physiological substrates (46). These enzymes alter membrane and receptor functions (47, 48) which in turn may change the cells' response to natural inducers of differentiation present in the serum used for the cell cultures (49, 51). Such possibilities may explain why ODCase inhibitors α-methylornithine and F_2MeOrn and the AdoMetDCase inhibitor MGBG did not change the differentiation patterns in both control and PMA-treated cells. If, on the other hand, the polyamine changes are simply a consequence of the induced differentiation, the fact remains that changes in their levels precede inhibition of growth, changes in cell appearance, and lysozyme activity. Thus, they may at least serve as early markers of differentiation in HL-60 cells and perhaps other cell types.

This research was sponsored jointly by the National Cancer Institute under Interagency Agreement 40-636-77, Environmental Protection Agency under Interagency Agreement 79-D-X0533, and the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

1. Hecker, E. (1978) in *Mechanisms of Tumor Promotion and Cocarcinogenesis*, eds. Slaga, T. J., Sivak, A. & Boutwell, R. K. (Raven, New York), pp. 14–48.
2. Berenblum, I. (1969) *Prog. Exp. Tumor Res.* **11**, 21–30.
3. Boutwell, R. K. (1974) *CRC Crit. Rev. Toxicol.* **2**, 419–447.
4. Cohen, R., Pacifici, M., Rubinstein, N., Beihi, J. & Holtzer, H. (1977) *Nature (London)* **266**, 538–540.
5. Rovera, G., O'Brien, T. G. & Diamond, L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2894–2898.
6. Yamasaki, H., Fibach, E., Nudel, U., Weinstein, I. B., Rifkind, R. A. & Marks, P. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3451–3455.
7. Diamond, L., O'Brien, T. G. & Rovera, G. (1977) *Nature (London)* **269**, 247–249.
8. Pacifici, M. & Holtzer, H. (1977) *Am. J. Anat.* **150**, 207–212.
9. Ishii, D. N., Fibach, E., Yamasaki, H. & Weinstein, I. B. (1978) *Science* **200**, 556–559.
10. Kasukabe, T., Honma, Y. & Hozmi, M. (1979) *Gann* **70**, 119–123.
11. Sukdeb, M. & Heidelberger, C. (1980) *Cancer Res.* **40**, 334–338.
12. Miao, R. M., Fieldsteel, A. H. & Fodge, D. W. (1978) *Nature (London)* **274**, 271–272.
13. Huberman, E., Heckman, C. & Langenbach, R. (1979) *Cancer Res.* **39**, 2618–2624.
14. Nakayasu, M., Shoji, M., Aoki, N., Sato, S., Miwa, M. & Sugimura, T. (1979) *Cancer Res.* **29**, 4668–4672.
15. Stuart, R. K. & Hamilton, J. A. (1980) *Science* **208**, 402–404.
16. Nagasawa, K. & Mak, T. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2964–2968.
17. Huberman, E. & Callaham, M. F. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1293–1297.
18. Rovera, G., O'Brien, T. G. & Diamond, L. (1979) *Science* **204**, 868–870.
19. Rovera, G., Santoli, D. & Damsky, C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2779–2783.
20. Lotem, J. & Sachs, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5158–5162.
21. Rovera, G., Olashaw, N. & Meo, P. (1980) *Nature (London)* **284**, 69–70.
22. Vorbrodt, A., Meo, P. & Rovera, G. (1979) *J. Cell Biol.* **83**, 300–307.
23. Soprano, K. J. & Baserga, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1566–1569.
24. Janne, J., Poso, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293.
25. Pegg, A. E. & Williams-Ashman, H. G. (1980) in *Polyamines in Biology and Medicine*, eds. Morris, D. R. & Morton, L. (Dekker, New York), in press.
26. Mamont, P., Bohlen, P., McCann, P. P., Bey, P., Schuber, F. & Tardif, C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1626–1630.
27. Newton, N. E. & Abdel-Monem, M. M. (1977) *J. Med. Chem.* **20**, 249–253.
28. O'Brien, T. G., Simsiman, R. C. & Boutwell, R. K. (1975) *Cancer Res.* **35**, 2426–2433.
29. Emanuelsson, H. & Heby, O. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1039–1042.
30. Bolander, F. F. & Topper, Y. J. (1979) *Biochem. Biophys. Res. Commun.* **90**, 1131–1135.
31. Tukigawa, M., Ishida, H., Takano, K. & Suzuki, F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1481–1485.
32. Gazitt, Y. & Friend, C. (1980) *Cancer Res.* **40**, 1727–1732.
33. Collins, S. J., Ruscetti, F. W., Gallagher, R. E. & Gallo, R. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2458–2462.
34. Krystosek, A. & Sachs, L. (1976) *Cell* **9**, 675–684.
35. Weeks, C. E. & Slaga, T. J. (1979) *Biochem. Biophys. Res. Commun.* **91**, 1488–1496.
36. Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295.
37. Van-Duuren, B. L., Segal, A., Tseng, S.-S., Rush, G. M., Loewengart, G., Mate, U., Roth, D., Smith, A., Melchionne, S. & Seidman, I. (1978) *J. Med. Chem.* **21**, 26–31.
38. Forbes, P. D., Urbach, F. & Davies, R. E. (1979) *Cancer Lett.* **7**, 85–90.
39. Breitman, T. R., Selonick, S. E., & Collins, S. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2936–2940.
40. Mamont, P. S., Duchesne, M., Grove, J. & Bey, P. (1978) *Biochem. Biophys. Res. Commun.* **81**, 58–66.
41. Abdel-Monem, M. M., Newton, N. E. & Weeks, C. E. (1974) *J. Med. Chem.* **17**, 447–451.
42. Yuspa, S. H., Lichti, U., Ben, T., Patterson, E., Hennings, H., Slaga, T. J., Colburn, N. & Kelsey, W. (1976) *Nature (London)* **262**, 402–404.
43. Lichti, U., Slaga, T. J., Ben, T., Patterson, E., Hennings, H. & Yuspa, S. H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3908–3912.
44. O'Brien, T. G., Lewis, M. A. & Diamond, L. (1979) *Cancer Res.* **39**, 4477–4480.
45. Hollta, E. (1977) *Biochemistry* **16**, 91–100.
46. Folk, J. E., Park, M. H., Chung, S. I., Schrode, J., Lester, E. P. & Cooper, H. L. (1980) *J. Biol. Chem.* **255**, 3695–3700.
47. Levitzki, A., Willingham, M. & Pastan, I. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2706–2710.
48. Van Leuven, F., Cassiman, J.-J. & Van Den Berghe, H. (1980) *Cell* **20**, 37–43.
49. Kluge, N., Gaedicke, G., Steinheider, G., Dube, S. & Ostertag, W. (1974) *Exp. Cell Res.* **88**, 257–262.
50. Sachs, L. (1978) *Nature (London)* **274**, 535–539.
51. Stanley, E. R., Hensen, G., Woodcock, J. & Metcalf, D. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2272–2278.