

## Inhibition of DNA Synthesis of Melanoma Cells by Azelaic Acid

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Azelaic acid was successfully used in the clinical treatment of 7 cases of lentigo maligna in that remission of the lesions was observed in all our patients. In order to elucidate mechanism(s) of the beneficial clinical effects, we studied the effect of azelaic acid on cultured melanoma cells.

Cell numbers recovered from melanoma cell cultures grown for several days in the presence of 10 mM azelaic acid were 50–70% less than those recovered from control cultures or from cultures containing 10 mM adipic acid. This reduction of cell numbers was not due to a simple cytotoxic or cytolytic effect of azelaic acid but rather due to a dose-dependent inhibition of DNA synthesis. Interestingly, nontoxic concentrations of azelaic acid, which significantly reduced DNA synthesis of cultured melanoma cells, had no overt effect on the protein synthesis of these cells. It is conceivable that inhibition of DNA synthesis is one of the mechanisms by which azelaic acid prevents growth and proliferation of abnormal melanocytes.

Azelaic acid (1,7-heptanedicarboxylic acid, nonanedioic acid) is a straight-chained, saturated dicarboxylic acid. Based on the observation that C<sub>8</sub>–C<sub>13</sub> dicarboxylic acids act as competitive inhibitors of mushroom tyrosinase *in vitro* [1,2], Nazzaro-Porto et al used a 15% azelaic acid cream to topically treat lesions of lentigo maligna and observed clinical and histologic remission of the lesions in all patients studied [3,4]. These authors suggested that inhibition of tyrosinase activity was responsible for the *in vivo* effects of azelaic acid in lentigo maligna [3]. This assumption was challenged by studies of Pathak et al [5] in the guinea pig system, which demonstrated that azelaic acid represented only a poor depigmenting agent. Moreover, recent evidence exists [6,7] that (1) after incubation of melanoma cells with radioactively labeled dicarboxylic acids, the radioactivity is mainly incorporated into mitochondria and nuclei and that (2) C<sub>8</sub>–C<sub>12</sub> dicarboxylic acids may inhibit various mitochondrial enzymes. On the basis of all these observations one may argue that azelaic acid exerts its biologic effects via interaction with various intracellular targets. We therefore felt that simultaneous assessment of the effects of azelaic acid on various functional and phenotypical features of abnormal melanocytes might provide a better understanding of the beneficial effects of this compound in the treatment of disorders characterized by melanocyte dysfunction.

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### Abbreviations:

BSA: bovine serum albumin  
FCS: fetal calf serum  
LM: lentigo maligna  
MEM: minimum essential medium  
PAGE: polyacrylamide gel electrophoresis  
PBS: phosphate-buffered saline  
SDS: sodium dodecyl sulfate  
TCA: trichloroacetic acid

## MATERIALS AND METHODS

### *Clinicopathologic Studies*

Seven patients with lentigo maligna (LM) were treated twice daily by topical application of 20% azelaic acid cream (Schering AG, West Berlin, F.R.G.) to the area of the lesions for various time periods. Patient selection criteria included: (1) large size of lesion which would have necessitated a major surgical intervention; (2) histopathologic evidence obtained from a representative biopsy that the lesion was noninvasive (Clark level 1); (3) old age of patients (over 70 years). The study was approved by the Institutional Ethical Committee and informed consent was obtained from the patients.

In an attempt not to unreasonably reduce the size of the lesions studied and in order to prevent inflammatory stimuli possibly caused by multiple biopsies, biopsies were taken only before and after cessation of treatment and processed for histopathologic and, in some instances, electron microscopic studies.

### *Cell Culture*

Two melanoma cell lines, G-361 (human origin), and clone M-3 of the mouse Cloudman S-91 melanoma (S-91/M-3) were obtained from the American Type Culture Collection, Rockville, Maryland and grown in monolayer cultures for several passages in our laboratory before use. For comparative analysis the human lymphoblastoid Raji-cell line was propagated in our laboratory. Raji cells were maintained in medium RPMI 1640 (Flow Laboratories, Rockville, Maryland) whereas G-361 were grown in McCoy's 5a medium (Flow) and S-91/M-3 in Hams' F-10 medium (Flow). All media were supplemented with 10% heat-inactivated fetal calf serum (FCS) and gentamycin (Gibco, Grand Island, New York).

### *Assay for Cell Growth and Viability*

About  $2.5 \times 10^5$  cells were seeded in 25-cm<sup>2</sup> tissue culture flasks (Costar, Cambridge, Massachusetts) in 5 ml volumes. Azelaic acid (C<sub>9</sub>; nonanedioic acid, Schering AG) and for control purposes adipic acid (C<sub>6</sub>; hexanedioic acid, Serva, Heidelberg, F.R.G.) were dissolved in phosphate-buffered saline (PBS), adjusted to pH 7.4, and added to the freshly established cultures to give the desired final concentrations. At 24-h intervals, cells of triplicate cultures were mechanically detached with a rubber policeman, combined with the free-floating cells, and counted with a hemocytometer. Cell viability was assessed by trypan blue exclusion.

Adipic acid was chosen as a control drug since we wanted to compare the effects of azelaic acid with the effects of a substance of similar molecular configuration. Additionally, preliminary studies with a panel of dicarboxylic acids (C<sub>4</sub>–C<sub>10</sub>, data not shown) have shown that adipic acid is the longest-chained dicarboxylic acid with no profound effects on melanoma cell growth. In all experiments, either azelaic acid or adipic acid were continuously present throughout the entire culture period.

### *Protein- and DNA-Synthesis*

For determination of protein synthesis G-361 melanoma cells were harvested with a rubber policeman and were carefully washed. Cells ( $3 \times 10^6$  to  $5 \times 10^6$ ) were resuspended in 5 ml methionine-free minimum essential medium (MEM) (Gibco) containing 10% heat-inactivated, dialyzed FCS, glutamine, gentamycin, 5% McCoy's medium, and 1.25  $\mu$ Ci [<sup>35</sup>S]methionine (New England Nuclear, NEN, Boston, Massachusetts, 800–1000 Ci/mmol) and incubated with or without the respective dicarboxylic acids (final concentrations ranging from 10–40 mM) for 4 and 16 h, respectively, at 37°C/5% CO<sub>2</sub>. For the evaluation of DNA synthesis, G-361 melanoma cells were treated as described above with the exception that supplemented McCoy's medium with 35  $\mu$ Ci [<sup>3</sup>H]thymidine (NEN, 70–80 Ci/mmol) was used as incubation medium for internal labeling. [<sup>3</sup>H]Thymidine and dicarboxylic acids were added to

freshly split cells or to cells which were cultured for 20 h after splitting and incubation was performed for 4 and 8 h, respectively. After the respective incubation times, cells were harvested, washed, and the rate of protein and DNA synthesis was determined by measuring the incorporation of [<sup>35</sup>S]methionine and [<sup>3</sup>H]thymidine, respectively, into trichloroacetic acid (TCA)-precipitable material as described by Waithe and Hirschhorn [8]. All experiments were done in triplicates and values standardized on numbers of living cells. Standard deviations were always smaller than 15%.

For the evaluation of DNA synthesis by epidermal cells, C3H/He mouse epidermal cells were prepared by trypsinization of ear skin as described recently [9]. Cells ( $6 \times 10^6$ ) per determination were washed and suspended in 5 ml RPMI 1640 medium (Gibco), supplemented with 10% FCS, 35  $\mu$ Ci [<sup>3</sup>H]thymidine (70–80 Ci/mmol), and varying amounts of azelaic acid and adipic acid, respectively. After incubation at 37°C for 4 h, the cells were harvested and the DNA synthesis evaluated as described above.

#### Polyacrylamide Gel Electrophoresis (PAGE)

G-362 melanoma cells, incubated for 16 h at 37°C with [<sup>35</sup>S]methionine as described above, were lysed by a 2-min incubation in sodium dodecyl sulfate (SDS)-sample buffer (0.1 M Tris-HCl, pH 6.8; 4% glycerol, 2% SDS; 2% mercaptoethanol, 0.004% bromophenol blue) in a boiling-water bath. Following centrifugation 10- $\mu$ l aliquots were electrophoresed in a discontinuous electrophoresis system as described by Laemmli [10]. Gels were placed in a solution of 0.1% Coomassie Brilliant Blue R/10% acetic acid/50% methanol. After 16 h gels were destained in 15% acetic acid, 10% methanol for an additional 20 h, transferred to concentrated acetic acid, and then incubated in enhancer solution (10% 2,5-diphenyloxazole in acetic acid). Fluorographic analysis using Kodak X-Omat XS-5 film was done according to Laskey and Mills [11].

#### Cell-Free Protein Synthesis

Epidermal cell suspensions were prepared from trunk and ear skin from C3H/He mice (Zentralanstalt für Versuchstierzucht, Himberg, Austria) as recently described [9]. RNA was isolated from  $5 \times 10^8$  cells by phenol/chloroform and ethanol precipitation as described previously [12]. Yeast mRNA was purchased from NEN. Cell-free protein synthesis (1–5  $\mu$ g mRNA per sample) was carried out in the presence of [<sup>35</sup>S]methionine according to Pelham and Jackson [13] with reagents provided by NEN as previously described [14]. Following translation, 10  $\mu$ g bovine serum albumin (BSA) was added to each sample and proteins were precipitated by the addition of  $1 \times \text{vol}$  12% TCA, 0.1% methionine. The precipitates were collected on Whatman GF/C glass fiber filters and washed 6 times with 10 ml 6% TCA, 0.1% methionine. Filters were assayed for radioactivity in a Beckman liquid scintillation counter, Type 6800 after treatment with 80% Protosol (NEN).

## RESULTS

### Clinical Investigations

Topical treatment twice daily of 7 patients with histologically proved LM with a 20% azelaic acid cream resulted in complete clinical remission of LM lesions in all patients after 9–12 months of treatment (Fig 1A,B). Apart from a transitory, mild inflammatory reaction after initiation of therapy and some desquamation, treatment with azelaic acid was well tolerated and no side effects of either toxic or allergic nature were observed. Clinical improvement manifested as progressive diminution of the intensity of pigmentation and size of the lesion. Treatment was discontinued when complete disappearance of pigmented spots indicated clinical remission. At this time a second biopsy specimen was taken from the previously involved sites and processed for histopathology and electron microscopy. After cessation of azelaic acid treatment, patients were seen by two of us (H.P., K.W.) at bimonthly intervals. After follow-up observation periods ranging from 2–13 months, no signs of recurrence were disclosed in any of the 7 patients.

### Histopathology

The biopsy specimens from all 7 patients exhibited the diagnostic criteria of LM (Fig 1C). There was a proliferation of atypical melanocytes on the basal layer of the epidermis and

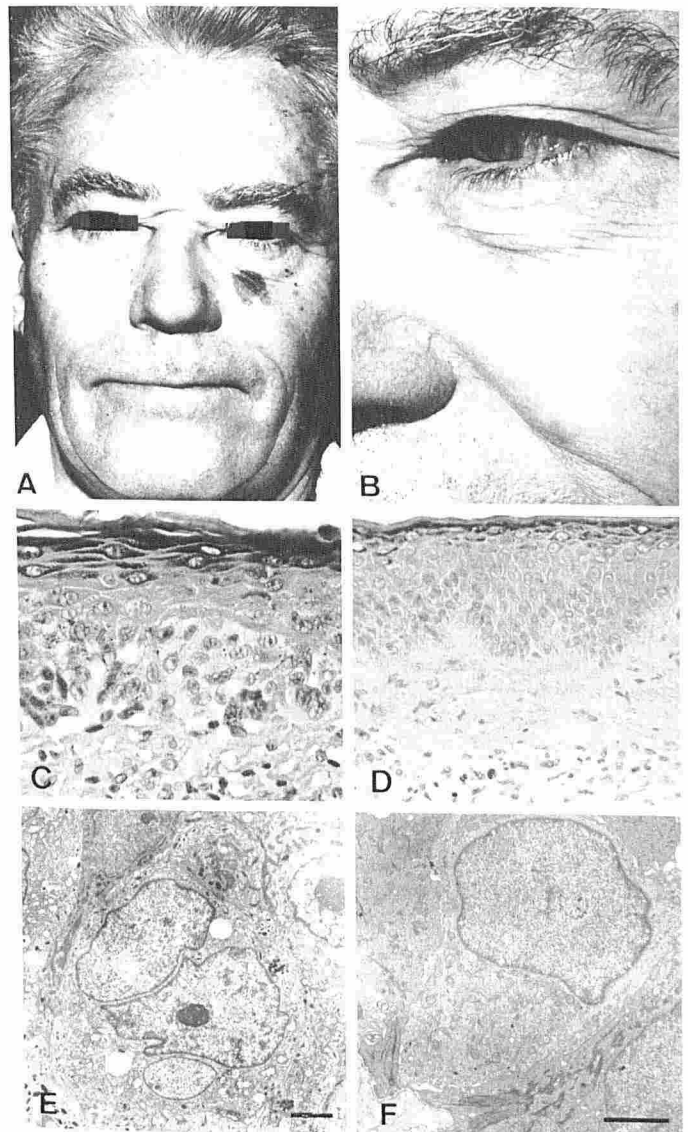


FIG 1. Patient J.K., male, age 75 years (1 of 7 representative cases). A, Clinical appearance—before treatment: coin-sized nonhomogeneous, brownish, irregularly shaped lentigo maligna on the left cheek. B, Clinical appearance—after treatment: complete resolution of the lesion after topical azelaic acid cream treatment for 9 months. C, Histopathology—before treatment: atypical melanocytes are seen both singly and in irregular nests in the basal layer of the epidermis. The papillary dermis contains a lymphohistiocytic infiltrate with some melanophages (H & E,  $\times 325$ ). D, Histopathology—after treatment: the biopsy from the previously involved skin shows a normally organized epidermis with slight hyperkeratosis, hypergranulosis, and acanthosis; a few melanophages are seen in the upper dermis (H & E,  $\times 250$ ). E, Electron microscopy—before treatment: the nucleus of a melanocyte is enlarged, shows deep indentations, and exhibits a prominent nucleus; an increased number of (morphologically abnormal) melanosomes is present in the cytoplasm ( $\times 7500$ ; bar = 1  $\mu$ m). F, Electron microscopy—after treatment: the nucleus of a melanocyte appears rounded and displays a regular chromatin pattern; the cytoplasm contains fewer organelles than seen in pretreatment specimens ( $\times 11,000$ ; bar = 1  $\mu$ m).

in the upper parts of the adnexal epithelium. The melanocytes—either spindle-shaped or more epitheloid in morphology—were seen both singly and in irregular nests; the upper parts of the epidermis were free from these cells. The papillary dermis showed solar elastosis and a dense irregularly distributed lymphohistiocytic infiltrate with a prominent number of melanophages. There were no tumor cells invading the dermis. After the treatment with azelaic acid (Fig 1D) the biopsy from

the previously involved skin showed a normally organized epidermis with slight hyperkeratosis and hypergranulosis and a moderate acanthosis. The melanocytes appeared normal in size and shape. Melanophages were still present in the papillary dermis.

#### Ultrastructural Findings

The electron microscopic changes in the lesions following treatment with azelaic acid were similar to those previously described [3]. Before azelaic acid treatment (Fig 1E), biopsies from LM lesions displayed an increased number of melanocytes of irregular size and shape seen singly and/or in small groups in the lower layers of the epidermis. The large nuclei of the melanocytes showed deep indentations and several nuclear profiles were frequently seen in one cell; the nucleoli were very prominent. The cytoplasm varied in its amount and in the composition of the organelles. Melanosomes and premelanosomes were present in the cytoplasm and in the dendritic process in an increased number; frequently they were morphologically abnormal in that they appeared in different sizes and exhibited a bizarre morphology including ring-forms. By contrast, posttreatment biopsies (Fig 1F) exhibited a strikingly different morphology. The melanocytes were normal in number and size. They occurred as singles and although sometimes more closely spaced, they were never clustered in groups. Their nuclei were round with a rather normal-appearing chromatin pattern, their cytoplasm appeared reduced in amount with fewer organelles in comparison to the pretreatment specimens. The melanosomes were still numerous and in all stages of maturation but abnormal structural variants were not seen. The surrounding keratinocytes appeared activated with many ribosomes and polyribosomes in their cytoplasm. The basal lamina exhibited multiple reduplications.

#### Effect of Azelaic Acid on Growth Kinetics

G-361 melanoma cell cultures were initiated at a seeding density of  $0.25 \times 10^6$  cells/culture flask. When grown either in medium alone or in the presence of 10 mM adipic acid, we regularly observed a 5- to 6-fold increase in cell numbers after 7 days of culture. In sharp contrast, cell numbers recovered from G-361 melanoma cell cultures grown in the presence of 10 mM azelaic acid were approximately 50–70% less than those recovered from melanoma cell cultures grown in either medium or 10 mM adipic acid. Azelaic acid (1 mM) or adipic acid (1 mM) had no overt effect on the cell numbers of G-361 melanoma cells (Fig 2A). At the initiation of the culture, G-361 melanoma cells were 70–90% viable as determined by trypan blue exclusion. Throughout the entire culture period (7 days) significant differences in viability among the various treatment groups were not encountered.

The adverse effect of 10 mM azelaic acid on the growth rate but not on cell viability of melanoma cells was even more pronounced when S-91/M-3 mouse melanoma cells were tested: starting cell numbers and recovered cell numbers after 2, 4, and 7 days of culture in the presence of 10 mM azelaic acid were

nearly identical. Again 1 mM azelaic acid or 10 mM and 1 mM adipic acid had no effect on the cell growth (Fig 2B). In order to determine the threshold of overt cytotoxicity of azelaic acid, G-361 melanoma cells were grown in azelaic acid concentrations ranging from 20–100 mM. Although melanoma cells grown in the presence of 20 or 40 mM azelaic acid were equally viable as the controls after 4 h of culture, differences in cell viability emerged after 48 h of culture. Whereas 20 mM azelaic acid did not overtly affect the cell viability, 40 mM or 100 mM azelaic acid resulted in a 70–99% decrease of cell viability as compared to control cultures (data not shown). Thus, clearly, azelaic acid action on cell viability is a time- and dose-dependent phenomenon.

In order to test the cell-type specificity of the inhibitory effect of azelaic acid on the growth rate of cells, lymphoblastoid Raji cells were grown in the presence or absence of 10 mM azelaic acid or adipic acid. In analogy to our observations with melanoma cells, neither azelaic acid nor adipic acid led to alterations in cell viability. However, after only 3 days of culture, cell numbers recovered from cultures containing 10 mM azelaic acid were 60–80% lower than those collected from control cultures i.e., medium alone or with 10 mM adipic acid (data not shown).

#### DNA Synthesis

In order to clarify whether the azelaic acid-induced reduction of cell numbers was preceded by an inhibition of DNA synthesis, either freshly split or 20-h precultured adherent G-361 human melanoma cells were incubated in the presence of [ $^3$ H]thymidine and dicarboxylic acids. After 8 h the [ $^3$ H]thymidine incorporation of precultured cells into TCA-precipitable material was inhibited in a dose-dependent fashion by azelaic acid. Adipic acid had a slight inhibitory effect but 4 times as much needs to be added to get a similar effect to azelaic acid (Fig 3). The inhibitory action of azelaic acid on DNA synthesis was also found after incubating freshly split G-361 melanoma cells for 4 h with azelaic acid at concentrations of 10–40 mM (Fig 4). Since significant differences in cell numbers were not observed until 12 h of cultivation of G-361 melanoma cells in the presence of dicarboxylic acid (data not shown), reduction of cell proliferation by azelaic acid is clearly preceded by a considerable inhibition of DNA synthesis. This finding supports the suggestion that azelaic acid acts primarily on DNA-dependent proliferative capacity of melanoma cells.

On the other hand, when cultures of murine epidermal cells were incubated for 4 h and tested for DNA synthesis capacity, we found no influence of azelaic acid and adipic acid, respectively, in concentrations up to 40 mM (Table I). However, it should be mentioned that in our primary murine epidermal cell cultures the DNA synthesis reaches a plateau between 4 and 16 h. Since one cannot exclude the possibility that a putative action of azelaic acid (or adipic acid or both) on DNA synthesis by keratinocytes would require the exposure of these cells to the drug(s) for more than 4 h, a possible effect of dicarboxylic acids on the de novo DNA synthesis would escape our detection.

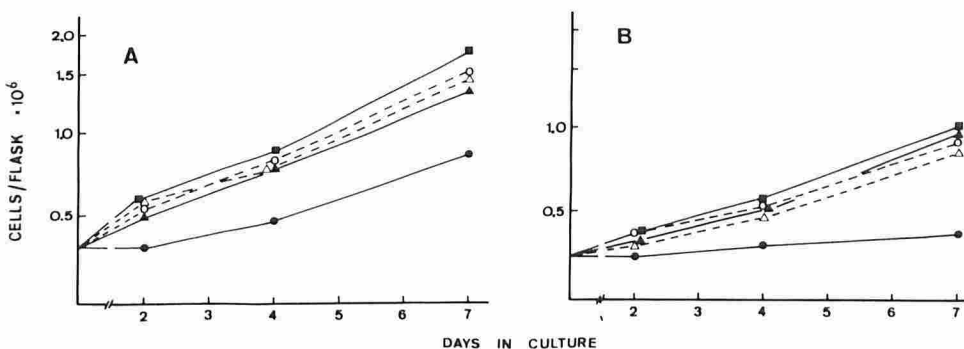


FIG 2. Growth kinetics of melanoma cell lines. Human G-361 (A) and mouse (S-91/M-3) (B) melanoma cells were grown in the presence or absence of various concentrations of azelaic acid or adipic acid and cell numbers were determined after distinct time intervals. Values are the means of triplicates of 1 of 3 representative experiments. ●—● = Azelaic acid  $10^{-2}$  M; ○—○ = azelaic acid  $10^{-3}$  M; ▲—▲ = adipic acid  $10^{-2}$  M; △—△ = adipic acid  $10^{-3}$  M; ■—■ = medium control.

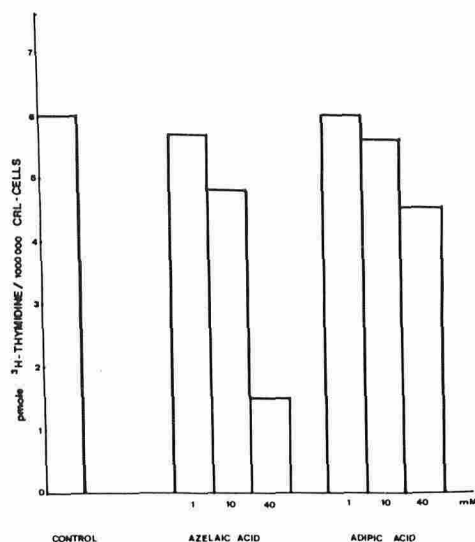


FIG 3. DNA synthesis of G-361 melanoma cells. G-361 melanoma cells were seeded in culture flasks and after 20 h various concentrations of azelaic acid and adipic acid, respectively, were added. After 8 h in the presence of [<sup>3</sup>H]thymidine, cells were harvested and the incorporation of [<sup>3</sup>H]thymidine into TCA-precipitable material was determined. Values are standardized on numbers of living cells and are the means of triplicates (SD less than 15%).

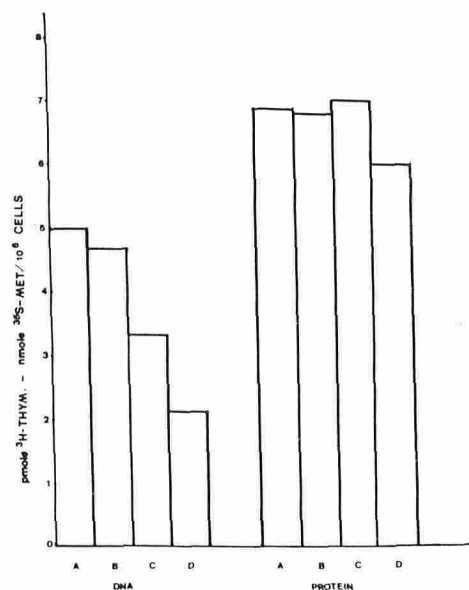


FIG 4. DNA and protein synthesis of G-361 melanoma cells. G-361 melanoma cells were freshly seeded and grown in medium containing [<sup>3</sup>H]thymidine (DNA synthesis) or [<sup>35</sup>S]methionine (protein synthesis) in the presence or absence of various concentrations of azelaic acid (C<sub>9</sub>) for 4 h. The incorporation of [<sup>3</sup>H]thymidine into DNA and [<sup>35</sup>S]methionine into protein, respectively, was determined. A = Control; B = 0.01 M C<sub>9</sub>; C = 0.02 M C<sub>9</sub>; D = 0.04 M C<sub>9</sub>.

#### Protein Synthesis

In order to find out whether azelaic acid concentrations that lead to a considerable inhibition of DNA synthesis also interfere with other major metabolic pathways, the influence of this dicarboxylic acid on protein synthesis of human melanoma cells was evaluated. No differences in the rate of the synthesis of total cell protein as measured by the incorporation of [<sup>35</sup>S]methionine into TCA-precipitable material was observed after a 4-h incubation of G-361-melanoma cells in the presence of various concentrations of azelaic acid (Fig 4). Moreover, neither azelaic acid nor adipic acid reduced the rate of protein synthesis

TABLE I. DNA synthesis of murine epidermal cells

		pmol [ <sup>3</sup> H]thymidine/ 10 <sup>6</sup> cells
Control		3.1 ± 0.3
Azelaic acid	10 mM	3.4 ± 0.2
	20 mM	3.2 ± 0.4
	40 mM	3.2 ± 0.5
Adipic acid	10 mM	3.2 ± 0.1
	20 mM	3.2 ± 0.3
	40 mM	3.1 ± 0.3

Murine epidermal cells were isolated by trypsinization [9]. After 6 h in the presence of [<sup>3</sup>H]thymidine and dicarboxylic acids, cells were harvested and the incorporation of [<sup>3</sup>H]thymidine into TCA-precipitable material was determined and standardized on numbers of living cells. Results are the means ± SD of 3 determinations.

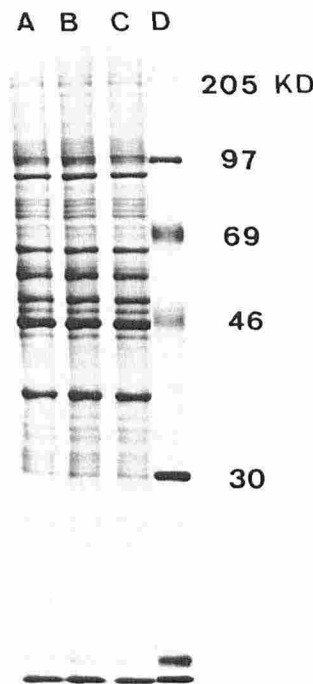


FIG 5. Protein synthesis of G-361 melanoma cells. G-361 melanoma cells were grown for 16 h in medium containing [<sup>35</sup>S]methionine in absence (A), and continuous presence of 10 mM azelaic acid (B) and adipic acid (C), respectively. Cells were harvested, lysed in SDS sample buffer, and analyzed by SDS-PAGE and fluorography as described in *Materials and Methods*. Molecular weight standards (D) were <sup>14</sup>C-labeled myosin (205 kD), phosphorylase B (97 kD), bovine serum albumin (69 kD), egg albumin (46 kD), and carbonic anhydrase (30 kD).

during a 12-h exposure of G-361 melanoma cells (control group 32.8 ± 0.4 nmol [<sup>35</sup>S]methionine/10<sup>6</sup> cells vs 31.0 ± 0.6 and 31.8 ± 1.6 nmol/10<sup>6</sup> cells for 20 mM azelaic acid and 20 mM adipic acid, respectively). Also, no influence of azelaic acid and adipic acid, respectively, on the patterns of biosynthetically labeled G-361 melanoma cell proteins could be detected by SDS-PAGE (Fig 5).

In addition, inability of azelaic acid and adipic acid to inhibit protein synthesis was further demonstrated by the use of a cell-free translation system. Using epidermal RNA and yeast mRNA, respectively, no inhibition of translation could be detected in the presence of dicarboxylic acids (Fig 6).

#### DISCUSSION

Confirming the observations of Nazzaro-Porro et al [3] a clinical remission of LM was observed in all our patients after 9–12 months of treatment. Remission of the lesions was accompanied by the disappearance of abnormal melanocytes as con-

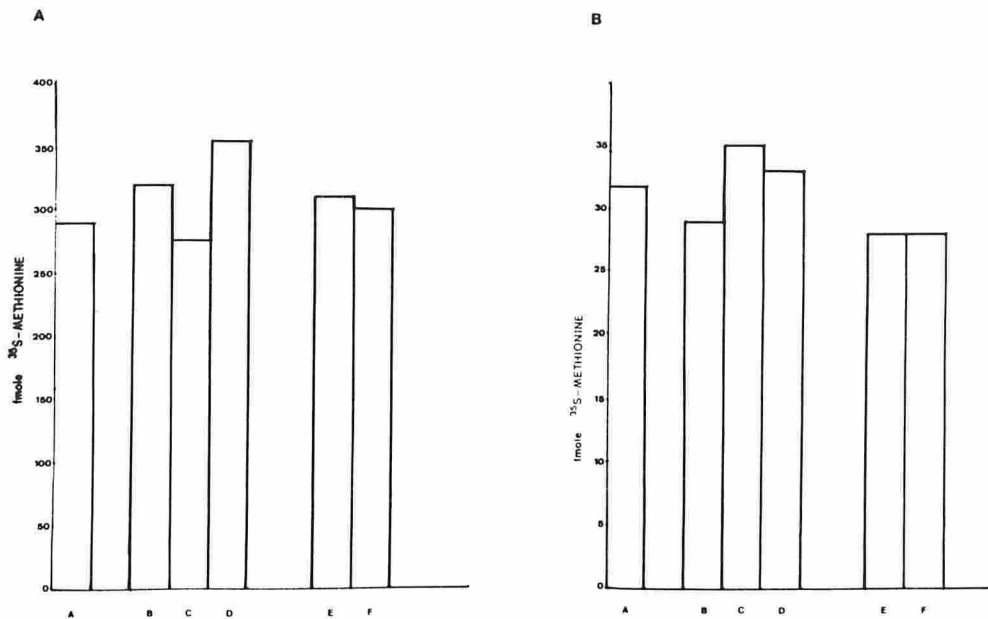


FIG 6. In vitro translation of mRNA. Yeast mRNA (A) or epidermal RNA (B) was translated in the presence of [<sup>35</sup>S]methionine in a rabbit reticulocyte system in the presence or absence of various concentrations of azelaic acid (C<sub>9</sub>) and adipic acid (C<sub>6</sub>), respectively. Protein synthesis was measured by determining the incorporation of [<sup>35</sup>S]methionine into TCA-precipitable material. Values are the means of duplicates (SD less than 10%). Columns A = control; B = 0.01 M C<sub>9</sub>; C = 0.001 M C<sub>9</sub>; D = 0.0001 M C<sub>9</sub>; E = 0.01 C<sub>6</sub>; F = 0.001 C<sub>6</sub>.

firmed by histopathology and by electron microscopy. Contrasting with this clinicopathologic phenomenon, there exist currently no conclusive data on the mechanism of the disappearance of abnormal melanocytes following azelaic acid treatment. The rather high doses (20%) of topically applied azelaic acid required to induce clinical remissions raise the question as to whether the azelaic acid effect is cytotoxic in nature. Such a cytotoxic effect may be either nondiscriminatory (acting on all cells) or, alternatively, may affect abnormal melanocytes in a selective fashion. The former possibility appears unlikely in that in our and other [3] clinicopathologic studies frank epidermal necrosis was never observed. The latter possibility which is favored by several investigators [3,15,16] cannot be excluded with certainty but appears unlikely since treatment periods from 9–12 months are required to induce complete clinicopathologic remission of the disease.

Since these observations indicate that cytolysis of malignant melanocytes is probably not responsible for the clinical effects seen, we established an in vitro system which would permit us to examine the effects of azelaic acid on melanoma cells under nontoxic conditions. We tested the effects of various concentrations of azelaic acid on viability and growth of two melanoma cell lines. Whereas azelaic acid concentrations ranging from 40–100 mM resulted in a sharp decline of cell viability within 48 h and were thus too high for further studies, concentrations of 10–20 mM did not overtly affect cell viability but led to a significant reduction in the growth rate of melanoma cells. Therefore it was reasonable to assume that—in a nontoxic dose range—azelaic acid leads to metabolic alterations causing decreased proliferation. In fact, our experiments clearly showed that 10–20 mM azelaic acid, but not adipic acid, when continuously present in melanoma cell cultures leads to a pronounced decrease in DNA synthesis without adversely affecting cell viability.

One might still argue at this point that the observed inhibition of DNA synthesis by azelaic acid does not represent a selective effect on DNA but rather reflects the impairment of other metabolic functions. However, at concentrations of 10–40 mM (concentrations which reduced DNA synthesis) azelaic acid had no measurable effect on the overall protein synthesis of human melanoma cells exposed for culture periods from 4–48 h. The possibility still exists that long-period incubation of melanoma cells with high (i.e., toxic) doses of azelaic acid might nonetheless result in an inhibition of both DNA and protein synthesis. This could lead to sudden cell death and might therefore explain our finding that 40–100 mM azelaic acid

results in a dramatic reduction in the viability of cultured melanoma cells. Since incorporation of radiolabeled amino acids into protein is standardized on numbers of living cells, sudden cell death due to a combined reduction of DNA and protein synthesis might explain why we failed to detect any effect of azelaic acid on protein synthesis even after long-period incubation.

The azelaic acid effect on the DNA synthesis and growth kinetics of melanoma cells is not specific for these cells as a similar antiproliferative effect was observed with Raji cells. By contrast, 10–40 mM azelaic acid does not inhibit DNA synthesis of cultured keratinocytes, which is in keeping with the observations that in vivo application of azelaic acid cream does not overtly influence the growth and differentiation of keratinocytes, as judged by clinical and histopathologic criteria. Assuming then that under in vivo conditions azelaic acid preferentially affects malignant melanocytes within the epidermis, one may speculate that this could result from enhanced uptake mechanisms operative in these cells. Cells susceptible to azelaic acid might possess enhanced diffusion uptake mechanisms or specific receptors for dicarboxylic acids in analogy to what has been recently reported for retinoids [17].

In summary, our data demonstrate that nontoxic concentrations of azelaic acid reduce melanoma cell growth in culture and that this effect is preceded and paralleled by an inhibition of DNA synthesis. We further speculate that a threshold concentration of azelaic acid is sufficient to arrest the growth of only abnormal, highly proliferative melanocytes which would result in a shortened in vivo life span and/or possibly, in a higher susceptibility of these cells to host defense mechanisms. This might in turn lead to the destruction of these cells and might explain the beneficial effects of azelaic acid on LM.

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