0022-202X/85/8503-0216$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 85:216–221, 1985

Vol. 85, No. 3
Printed in U.S.A.

Effect of Dicarboxylic Acids on Harding-Passey and Cloudman S91 Melanoma Cells in Tissue Culture*

ELVIRA J. ROBINS, PH.D., AODÁN S. BREATHNACH, M.D., BARBARA J. WARD, PH.D., YASH P. BHASIN, B.SC., LESLIE ETHRIDGE, F.I.S.T., MARCELLA NAZZARO-PORRO, M.D., SIRO PASSI, PH.D., AND MAURO PICARDO, M.D.

Department of Anatomy, St. Mary's Hospital Medical School, London, U.K., and Instituto Dermatologico S. Gallicano (MN-P, SP, MP), Rome, Italy

Clinically, dicarboxylic acids have a cytotoxic effect on the abnormally hyperactive and malignant epidermal melanocyte, and diacids from C_8 to C_{13} have been shown to inhibit mitochondrial oxidoreductases. Here, their effect on the growth kinetics and ultrastructure of murine melanoma cells in culture is examined.

Cultures of Harding-Passey and Cloudman S91 melanoma cells were exposed to single doses of the disodium salts of C_{12}, C_9, and C_6 (which does not significantly inhibit mitochondrial enzymes) dicarboxylic acids at concentrations of 10^{-3} M to 10^{-1} M. With C_{12} and C_9, viability and cell proliferation over 3 days were significantly affected by concentrations greater than 10^{-2} M. With exposure to C_6 at 10^{-1} M and to medium to which NaCl was added to produce equal osmolarity, the effect was much less. Electron microscopy of cells exposed to C_9 at 10^{-1} M for 1 h and 6 h revealed massive swelling of mitochondria with destruction of cristae, but plasma and nuclear membranes and membranes of endoplasmic reticulum were intact. Similar damage was not seen with C_6 at 10^{-1} M nor with equiosmolar NaCl.

The results confirm (1) the cytotoxicity of dicarboxylic acids for malignant melanocytes, and (2) that the mitochondrion is a prime target for their action.

The dicarboxylic acids, azelaic acid and dodecanedioic acid, applied topically in a 20% (1 M) cream have a beneficial effect on hyperpigmentary disorders such as chloasma and lentigo maligna, and can cause regression of primary lesions of malignant melanoma [1–5]. In the latter two conditions, degeneration and disappearance of abnormal melanocytes were observed on histologic and ultrastructural examination during treatment, and a similar effect was reported by Ertle, Wiskemann, and Jänner [6] on melanocytes of malignant lentigenes in a patient with xeroderma pigmentosum.

Recent biochemical [7] and analytical ultrastructural autoradiographic investigations of ours [8,9] have provided evidence that inhibition of mitochondrial oxidoreductases, and possibly of nuclear DNA synthesis, may be a major factor in the cytotoxic effect of dicarboxylic acids. In line with these results, murine melanoma cells in tissue culture have been exposed to different concentrations of the sodium salts of azelaic (C_9), dodecanedioic (C_{12}), and adipic (C_6) acids, and the effect on viability and cell proliferation as compared with controls was observed. Cells of cultures in the different circumstances were examined ultrastructurally for any evidence of damage to plasma membrane, nuclei, mitochondria, or other organelles.

Manuscript received November 9, 1984; accepted for publication March 1, 1985.

* Part of this work was presented at the 14th Annual Meeting of the European Society for Dermatological Research, Amsterdam, May 1984.

This work was supported by grants from The Cancer Research Campaign, Schering AG, Berlin, the Wellcome Trust, and by Grant No. 820036796, Control of Tumor Growth, C.N.R., Italy.

Reprint requests to: Prof. A. S. Breathnach, Department of Anatomy, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, U.K.

Abbreviations:
C_{12}: dodecanedioic acid
C_9: azelaic acid
C_6: adipic acid

MATERIALS AND METHODS

Cell Cultures

Cultures were grown of Harding-Passey and Cloudman S91 murine melanomata. The basic culture media were as previously described [9], and were supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. In order to exclude possible mycoplasma contamination, the fetal calf serum used was guaranteed mycoplasma-free, manual pipettes were used throughout, and regular staining with the fluorescent DNA-binding benzimidole derivative Hoechst 33258 [10] was carried out. The cells were grown in plastic Petri dishes (diameter 3.5 cm), and all the cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2, 95% air.

Dicarboxylic Acids

The dicarboxylic acids used were dodecanedioic acid (C_{12}), azelaic acid (C_9), and adipic acid (C_6). Adipic acid does not significantly inhibit mitochondrial respiration in isolated rat liver mitochondria, even at 3×10^{-2} M [7], and its use therefore could serve as an additional control for any effect due to the C_9 and C_{12} diacids. Since the acids themselves are not sufficiently soluble in culture medium to produce the range of concentrations required, their disodium salts were used. A 1 M solution of the disodium salt of the diacid was prepared in each case by heating together the diacid, NaOH, and water in stoichiometrically equivalent quantities. The pH was adjusted to 7 in the case of each individual diacid used before the final dilution with distilled water was made. The appropriate volume of the C_9 salt solution ($C_9$2Na) was added to the culture medium to give the following concentrations: 10^{-3} M, 10^{-2} M, and 10^{-1} M. The sodium salt of the C_{12} diacid (C_{12}2Na) was not soluble in culture medium at 10^{-1} M, and so was used at 10^{-3} M, 10^{-2} M, and 5×10^{-2} M. The C_6 diacid salt ($C_6$2Na) was used at the highest concentration of the $C_9$2Na, i.e., 10^{-1} M. The pH of all the media with added diacids was within physiologic range (Table I). Diacid salts at each concentration were applied in the appropriate culture medium to each cell line.

It was considered necessary to test whether adding the diacid salts to the medium had an osmolar effect on the cell cultures over and above any specific effect. In order to achieve this, Cloudman cells were also grown either in the presence of 10^{-1} M $C_9$2Na, 10^{-1} M $C_6$2Na, or a control medium containing sodium chloride (7.02 g/liter). The latter produced an osmolarity of the medium (524 mOsmol/kg H_2O) equal to that containing the highest concentration of diacid salt used, i.e., 10^{-1} M, with little change in pH (see Table I).

In order to assess a possible effect of free sodium ions on proliferation, the ionic conductivity of medium alone, medium with 10^{-1} M $C_6$2Na, 10^{-1} M $C_9$2Na, and equiosmolar medium with NaCl was measured (Table I).

Viability Test

$C_9$2Na was applied in culture medium at concentrations of 10^{-1} M, 10^{-2} M, and 10^{-3} M to Cloudman cells. The cells were harvested after 1, 2, and 3 days. A control series of samples was also taken. Cells that

rounded up and became detached during the experiment and cells that remained attached to the dishes after the removal of the culture medium were separately tested for viability using trypan blue exclusion tests, and were replated in control medium and incubated for 2 days.

Cell Counts of Attached Cells

Cells were grown in 5-ml wells (diameter 3.5 cm) and these were set up with an inoculum which produced a cell count of 1.5–3.4 × 10^5 cells/ml (Cloudman), and 0.73–2.3 × 10^5 cells/ml (Harding-Passey) on the following day (day 0 of the experiment). This figure is referred to as the initial inoculum. Consistency of cell numbers was checked on day 0, prior to the addition of the diacids, by counting cells from 3 randomly selected wells, to establish the initial inoculum for each experiment.

Two runs of the following procedure for attached cells were carried out for both Harding-Passey and Cloudman lines. On day 0, diacid salts were applied in appropriate culture media in the concentrations stated (see "Dicarboxylic Acids" above) to the cultures. Attached cells were harvested and counted in a Coulter counter after 1, 2, or 3 days of growth. Three counts per well were carried out for each run, and appropriate counting controls without cells were carried out.

TABLE I. *Osmolarity, pH, and ionic conductivity of culture media with added sodium salts of C_6, C_9, C_{12}, and NaCl*

Solution	Osmolarity (mOsmol/kg H_2O)	Ionic conductivity (m Mhos)	pH
Cloudman medium alone	309	13.3	7.2
Cloudman medium + 10^{-1} M C_6	513	18.8	6.9
Cloudman medium + 10^{-1} M C_9	517	18.1	7.0
Cloudman medium + 5 × 10^{-2} M C_{12}	311	14.5	7.2
Cloudman medium + equiosmolar NaCl	524	23.8	7.3
Harding-Passey medium alone	312	13.3	7.2
Harding-Passey medium + 10^{-1} M C_6	517	19.6	7.0
Harding-Passey medium + 10^{-1} M C_9	520	18.9	7.1

Analysis of Counts

Graphs were prepared showing growth rate of cells as a percentage of the initial inoculum under the different experimental conditions (Figs 1–3). The raw data of counts were also analyzed by Student's *t*-test with the addition of Bessels's correction [11] to produce a *p* value for each daily experimental result compared with that day's control figure (Tables II–V).

Morphologic Studies

Confluent cultures of cells were incubated with each of the diacid salts for 1 h or 6 h. Control cultures (1) without the addition of diacid salt, and (2) with added sodium chloride to produce osmolarity equal to that of medium with added 10^{-1} M diacid salts, were also taken for morphologic studies after the same times. After incubation, the medium was removed, the cells were washed in phosphate-buffered saline and fixed for 5 min at room temperature in 2.5% glutaraldehyde in 0.2 M cacodylate buffer. After postfixation in osmium tetroxide and dehydration in 70% ethanol, the cells were gently scraped off the coverslips using a rubber spatula and centrifuged in ethanol at 25 *g* to form a pellet which was further processed for electron microscopy.

RESULTS

Viability Test

Cells that remained attached in the presence of diacid salts excluded trypan blue, indicating their viability. The detached cells stained positively with trypan blue and those that were replated with control medium did not reattach and grow within 2 days, indicating nonviability. The following results relate only to attached, viable cells.

Cell Counts of Attached Cells

All the diacid salts used altered the number of viable cells of both lines from control values over the 3 days of the experiments.

$C_9$2Na: With $C_9$2Na at 10^{-1} M, the number of viable, attached cells of both cell lines fell rapidly to just less than 40% of the initial inoculum for Harding-Passey, and 50% for Cloudman on day 1 (Fig 1*a*,*b*). Over the following 2 days, a drop by a further 20% occurred in Cloudman cell numbers, while the Harding-Passey numbers increased by approximately 20%, i.e., still less than 60% of the initial inoculum. The drop in cell numbers as compared with controls was significant throughout (Table II).

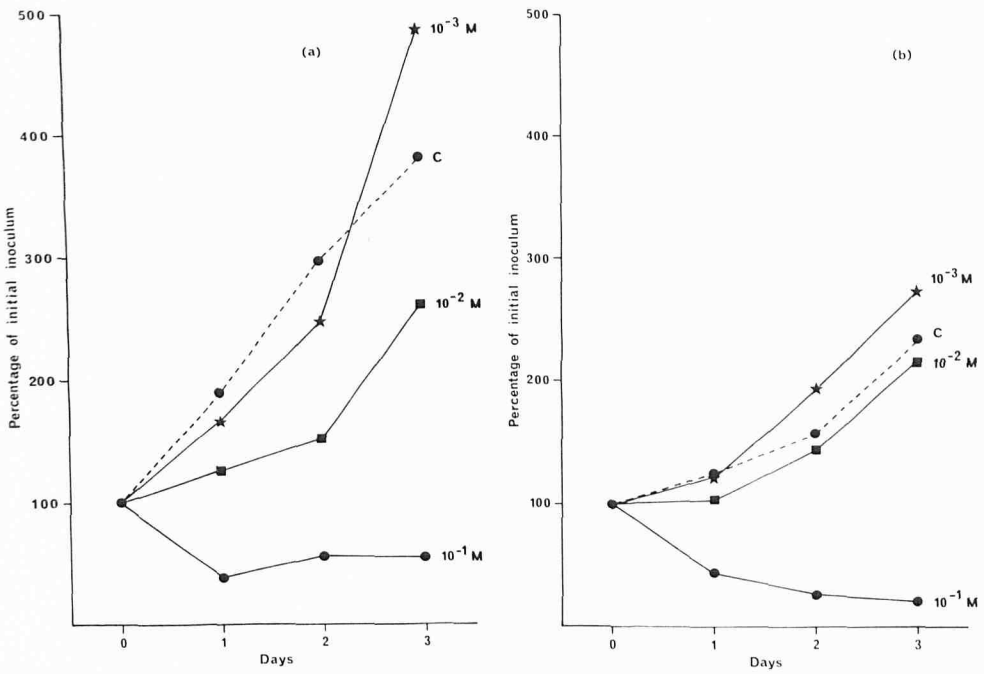

FIG 1. The effect of $C_9$2Na on (*a*) Harding-Passey and (*b*) Cloudman murine melanoma cells in culture. Cell counts on each of the 3 days of the experiment are expressed as a percentage of the initial inoculum counted on day 0. ●——● = 10^{-1} M, ■——■ = 10^{-2} M, ★——★ = 10^{-3} M, ●-----● = control, medium alone. See *p* values in Table II for significance.

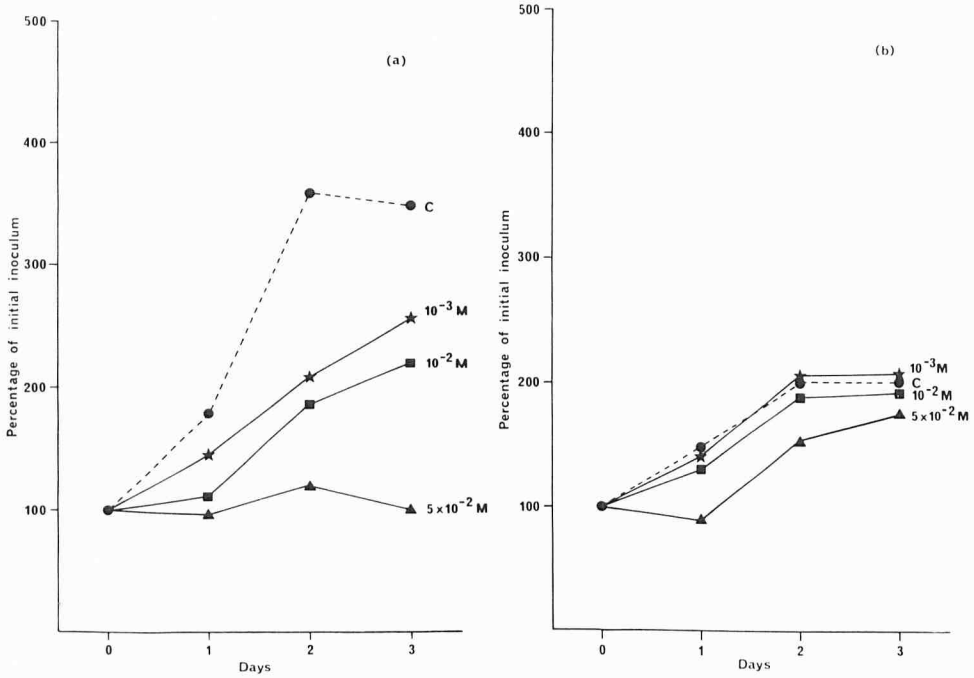

FIG 2. The effect of $C_{12}2Na$ on (*a*) Harding-Passey and (*b*) Cloudman murine melanoma cells in culture. Cell counts on each of the 3 days of the experiment are expressed as a percentage of the initial inoculum counted on day 0. ▲——▲ = 5×10^{-2} M, ■——■ = 10^{-2} M, ★——★ = 10^{-3} M, ●-----● = control, medium alone. See *p* values in Table III for significance.

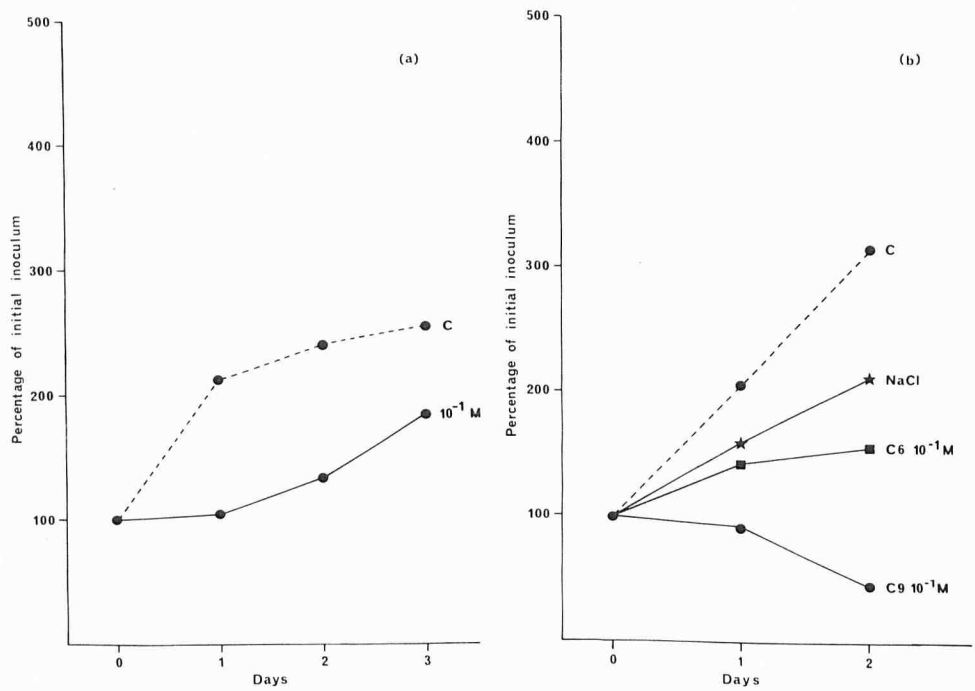

FIG 3. *a*, The effect of $C_6$2Na on Harding-Passey murine melanoma cells in culture. Cell counts on each of the 3 days of the experiment are expressed as a percentage of the initial inoculum on day 0. See *p* values in Table IV for significance. *b*, The effect on Cloudman murine melanoma cells in culture of added 10^{-1} M $C_9$2Na, $C_6$2Na, and of NaCl to produce an equal osmolarity of the culture medium (0.524 mOsmol/kg H_2O). Note greater effect of diacid salts, and significantly greater effect of C_9 as compared with C_6. See also Figs 1*b* and 3*a*. ●——● = 10^{-1} M $C_9$2Na, ■——■ = 10^{-1} M $C_6$2Na, ★——★ = NaCl, ●-----● = control, medium alone. See *p* values in Table V for significance.

TABLE II. *p Values for $C_9$2Na on Fig 1a and b*

Days of drug	Conc of drug (M)	Cloudman		Harding-Passey	
		Exp I	Exp II	Exp I	Exp II
1	10^{-1}	<.001	<.05	<.001	<.001
	10^{-2}	<.01	<.5 (NS)	<.01	<.001
	10^{-3}	<.01	<.01	<.5 (NS)	<.5 (NS)
2	10^{-1}	<.001	<.001	<.001	<.001
	10^{-2}	<.05	<.001	<.001	<.001
	10^{-3}	<.01	<.01	<.02	<.02
3	10^{-1}	<.001	<.001	<.001	<.001
	10^{-2}	<.001	<.5 (NS)	<.01	<.01
	10^{-3}	<.001	<.01	<.01	<.01

Values less than .05 are significant. NS = not significant.

TABLE III. *p Values for C_{12}2Na on Fig 2a and b*

Days of drug	Conc of drug (M)	Cloudman		Harding-Passey	
		Exp I	Exp II	Exp I	Exp II
1	5×10^{-2}	<.001	<.001	<.001	<.001
	10^{-2}	<.5 (NS)	<.05	<.001	<.001
	10^{-3}	<.5 (NS)	<.1 (NS)	<.01	<.001
2	5×10^{-2}	<.01	<.5 (NS)	<.001	<.001
	10^{-2}	<.05	<.01	<.001	<.001
	10^{-3}	<.05	<.001	<.001	<.001
3	5×10^{-2}	<.001	<.01	<.001	<.001
	10^{-2}	<.1 (NS)	<.05	<.001	<.001
	10^{-3}	<.01	<.01	<.001	<.001

Values less than .05 are significant. NS = not significant.

With 10^{-2} M, while continuous increase in viable cell numbers of both cell lines took place, with the Harding-Passey cells this increase was significantly less (Fig 1*a*; Table II) than with the control cells on all 3 days. With Cloudman cells, there was a statistically significant reduction in cell numbers at 10^{-2} M on day 2 compared with the control, and on days 1 and 3 numbers were significantly reduced in one run but not in the second run.

At a concentration of 10^{-3} M, an initial slight reduction in cell numbers occurred with both lines but by the third day the numbers had increased to overtake those of the controls and were significantly greater (Fig 1*a*,*b*; Table II).

$C_{12}2Na$: At all three concentrations used, $C_{12}2Na$ significantly reduced the number of viable Harding-Passey cells during the 3 days of the experiment as compared with controls (Fig 2*a*; Table III). At 5×10^{-2} M, cell numbers remained at approximately the initial inoculum level over the 3 days, while at 10^{-2} M and 10^{-3} M there was a steady increase in viable cell numbers. In the Cloudman cultures with 5×10^{-2} M $C_{12}2Na$, cell numbers by day 1 were significantly (15%) less than the initial inoculum. Cell numbers increased over the subsequent 2 days and in all but one instance were significantly less than control. At 10^{-2} M and 10^{-3} M, cell numbers were not consistently significantly different from controls (see Table III).

$C_6 2Na$: With $C_6 2Na$ at 10^{-1} M, there was a significant reduction in cell numbers of both lines over day 1, but they did not fall below those of the original inoculum (only Harding-Passey illustrated, Fig 3*a* and Table IV). This latter feature was in marked contrast to the effect of the 10^{-1} M $C_9 2Na$. Over the following 2 days, cell numbers progressively increased, and by

TABLE IV. *p Values for $C_6 2Na$ on Fig 3a*

Days of drug	Conc of drug (M)	Harding-Passey	
		Exp I	Exp II
1	10^{-1}	<.001	<.001
2	10^{-1}	<.001	<.001
3	10^{-1}	<.001	<.001

Values less than .05 are significant.

TABLE V. *p Values for Fig 3b*

Day	Exp I			Exp II		
	$C_9 2Na$[a]	$C_6 2Na$[b]	NaCl[c]	$C_9 2Na$[a]	$C_6 2Na$[b]	NaCl[c]
1	<.001	<.001	<.001	<.001	<.01	<.5 NS
2	<.001	<.01	<.01	<.001	<.01	<.01

Values less than .05 are significant. NS = not significant.
[a] $C_9 2Na$ 10^{-1} M.
[b] $C_6 2Na$ 10^{-1} M.
[c] NaCl equiosmolar to $C_9 2Na$ 10^{-1} M.

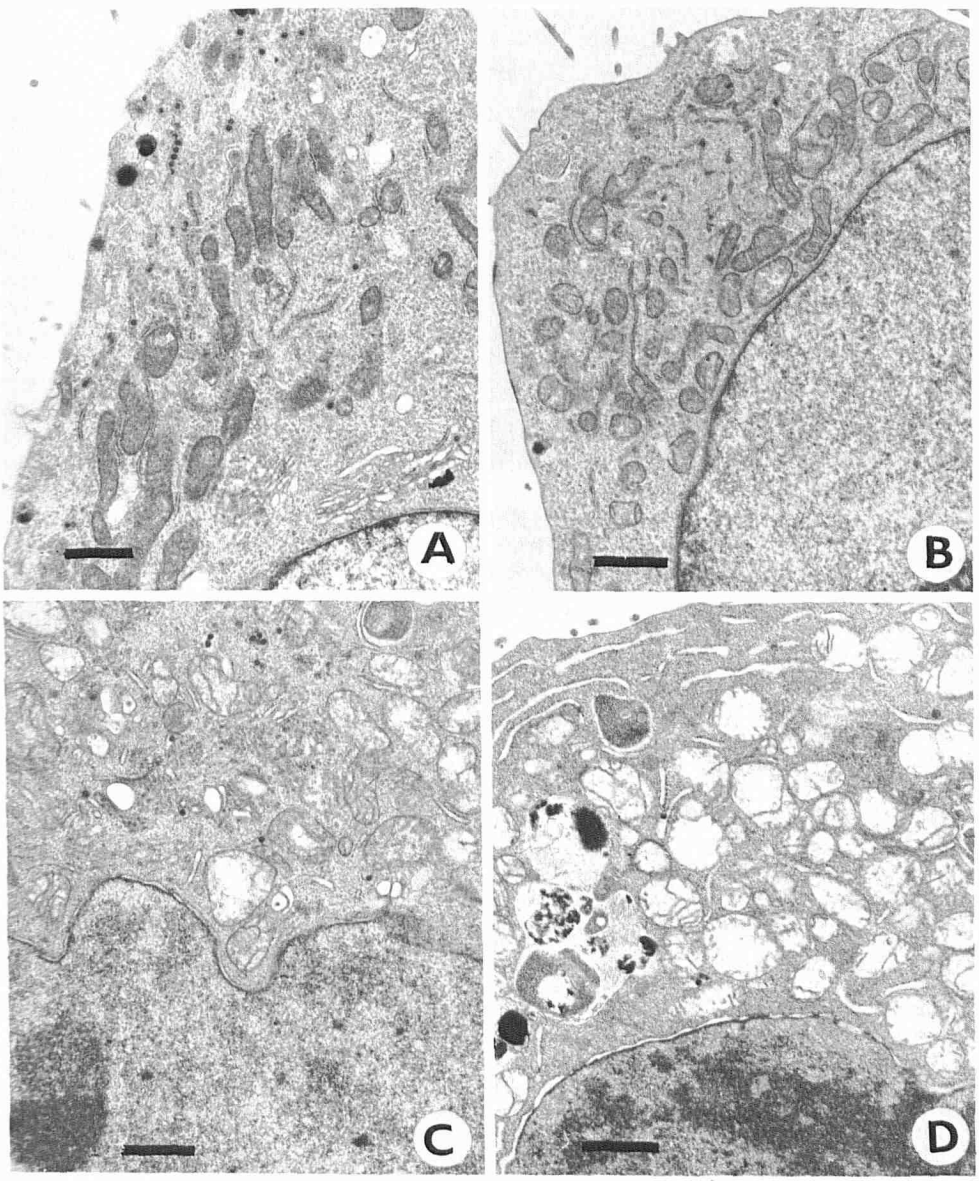

FIG 4. *A*, Control Harding-Passey melanoma cell. *B*, Harding-Passey melanoma cell exposed to disodium salt of adipic acid (C_6) at 10^{-1} M for 6 h in culture. Mitochondria are practically unaffected. *C*, Harding-Passey melanoma cell exposed to disodium salt of azelaic acid (C_9) at 10^{-1} M for 1 h. Note swelling and disruption of mitochondria. *D*, Harding-Passey melanoma cell exposed to disodium salt of azelaic acid (C_9) at 10^{-1} M for 6 h. Note massive swelling and disruption of mitochondria. All at × 9300.

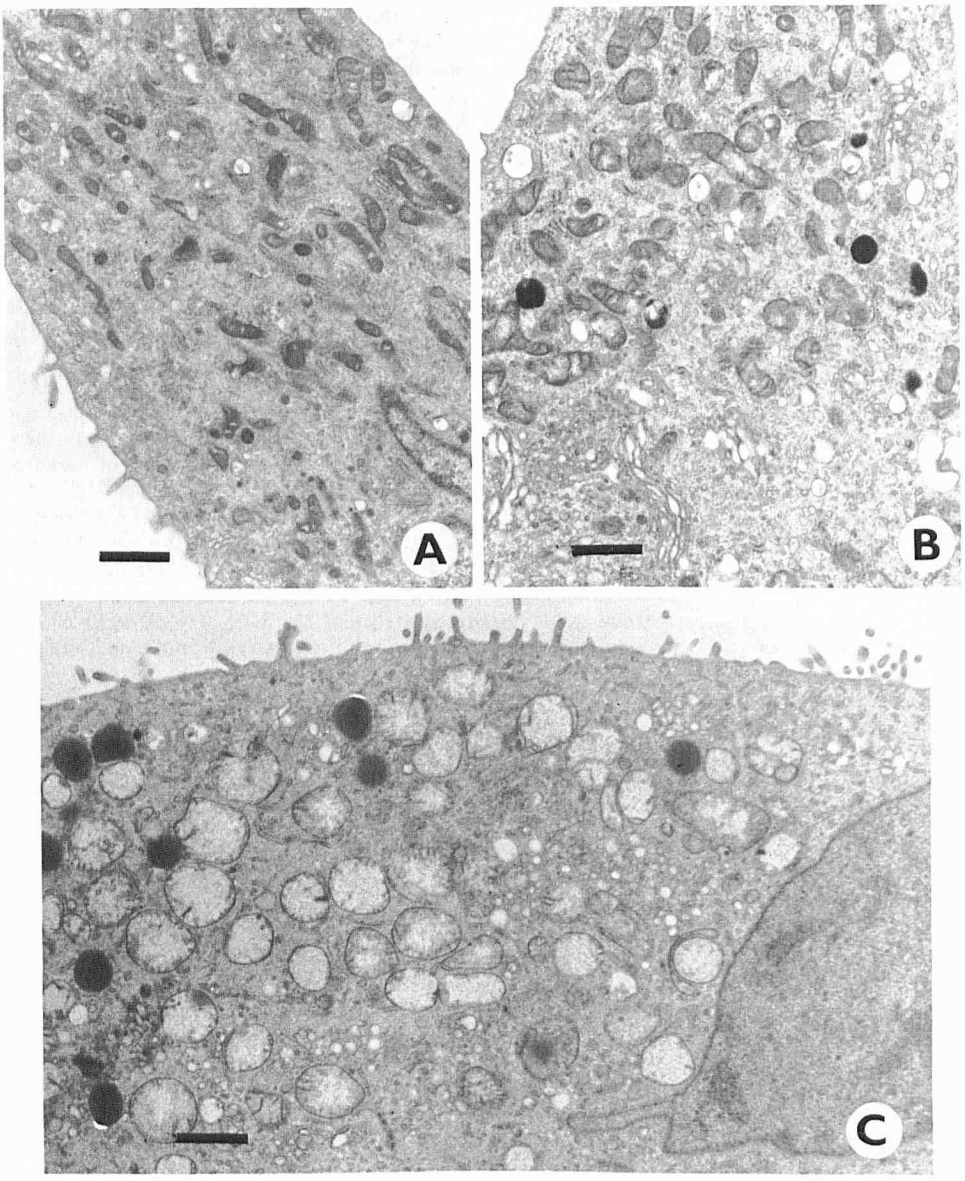

FIG 5. *A*, Control Cloudman melanoma cell. *B*, Cloudman melanoma cell exposed to NaCl of equal osmolarity to 10^{-1} M $C_9$2Na for 6 h in culture. Some slight swelling of mitochondria is evident. *C*, Cloudman melanoma cell exposed to 10^{-1} M $C_9$2Na for 6 h. Note massive swelling and disruption of mitochondria and presence of lipid droplets. All at × 9300.

day 3 the number of cells had almost doubled as compared with the initial inoculum.

Osmolarity

Fig 3*b* shows that the addition of NaCl to culture medium to produce a final osmolarity equivalent to that resulting from the addition of the two diacids at 10^{-1} M significantly reduced the number of viable cells over 2 days as compared with controls (Table V). With $C_6$2Na the reduction was significantly greater than with NaCl, but by day 2 cell numbers had increased by 50% of the initial inoculum. In contrast, with $C_9$2Na the number of cells dropped right from the start to less than 50% of the initial inoculum by day 2.

Morphologic Studies

Cells of cultures of both lines exposed to 10^{-3} M and to 5×10^{-2} M of $C_9$2Na and C_{12}2Na for up to 6 h, exhibited no detectable damage to mitochondria or other organelles. With $C_9$2Na at 10^{-1} M for 1 h, definite swelling of mitochondria was observed, but no other morphologic effect was seen (Fig 4*c*). After 6 h exposure, there was massive swelling of mitochondria with disruption of cristae and vacuolation, and lipid droplets were also prominent in the cytoplasm (Figs 4*d*, 5*c*). The plasma and nuclear membranes were invariably intact, and no morphologic damage to cytoplasmic organelles or other membranes was observed. No effects were observed with $C_6$2Na before 10^{-1} M for 6 h. With this concentration and time, some swelling of mitochondria of some cells was observed (Fig 4*b*), but a high proportion of cells was more or less indistinguishable from controls. Mitochondrial damage with $C_6$2Na at 6 h was certainly less than with $C_9$2Na at 1 h, and nothing comparable to the massive destruction seen with $C_9$2Na was observed.

Cells of cultures to which NaCl was added to produce an osmolarity similar to that of cultures with added diacid salts at concentration of 10^{-1} M, exhibited only minor swelling of mitochondria (Fig 5*b*), and no other observable damage.

DISCUSSION

This study has shown that the disodium salts of C_9 and C_{12} dicarboxylic acids, when added to cultures of Cloudman and Harding-Passey murine melanomata, at concentrations $\geq 10^{-2}$ M, have a positive inhibitory effect on cell proliferation over 3 days, and that at 10^{-1} M the C_9 diacid salt has a markedly adverse effect on cell viability. The C_6 diacid salt at 10^{-1} M initially depressed cell proliferation, but not nearly to the same extent as the C_9 and C_{12} diacids, though by the third day cell numbers were still significantly below those of the control. With 10^{-1} M $C_6$2Na there was no effect on cell viability.

The depression of cell proliferation could be attributed to the combined effects of a number of factors. Firstly, to the effect

of the higher osmolarity of the experimental media; secondly, to the presence of higher concentrations of Na ions in the experimental media as compared with controls, due to dissociation of the diacid salts; thirdly, to the presence of particular dicarboxylic ions in the experimental media exerting a separate and specific effect. The pH of media under various experimental conditions was essentially the same as the controls, so this could not be a factor.

The culture medium with added NaCl was equiosmolar to media to which 10^{-1} M $C_9$2Na and 10^{-1} M $C_6$2Na were added, and had a higher osmolarity than the control medium while the pH was similar. It can therefore serve as a control for any effect of increased osmolarity per se on the growth pattern of cells in the 10^{-1} M experimental media. This medium had a higher ionic conductivity and therefore a higher concentration of Na ions than the control medium or those to which 10^{-1} M $C_9$2Na and 10^{-1} M $C_6$2Na were added (see Table V). Thus, any effect observed on cultures with NaCl in the medium could be attributed to the first two factors listed above.

Cells grown in medium to which NaCl was added proliferated, but their numbers were significantly less than those of control cultures on both days 1 and 2. This indicates an effect due to high osmolarity and Na ion concentration. By comparison, the effect on cell numbers was significantly greater with $C_6$2Na 10^{-1} M of equal osmolarity but lower Na ion concentration (see Fig 3*b*, Table V). This in turn indicates an additional specific effect of the C_6 ion. With $C_9$2Na, equal in osmolarity and Na ion concentration to $C_6$2Na, the number of cells dropped right from the start to less than 50% of the initial inoculum, indicating an even more pronounced effect of the C_9 ion. With $C_9$2Na, in addition, cells became detached, and over the first day death was the predominant feature. Over the subsequent 2 days, numbers of viable cells diminished progressively until a static level of approximately 20% (Cloudman) and 50% (Harding-Passey) of the initial inoculum was reached (Fig 1*a*,*b*). Clearly, by comparison with the results of the NaCl and $C_6$2Na experiments, these effects are greater than those which higher osmolarity and greater Na ion concentration would be expected to produce. Similarly, the depressing effect on cell numbers of C_{12}2Na at 5×10^{-2} M cannot be due entirely to high osmolarity and/or an excess of Na ions. Here, both these factors were lower than with the 10^{-1} M $C_6$2Na medium, but there was a considerably greater drop in cell numbers (Figs 2*a*, 3*a*). One may conclude, therefore, that the C_9 and C_{12} diacid ions themselves are directly responsible for cell death and depression of proliferation.

Previous experiments have shown that dicarboxylic acids are metabolized via the β-oxidative pathway in the mitochondrial matrix [12], and electron autoradiographic studies using labeled C_{12} have shown the presence of the diacid and its breakdown products in the mitochondria [9]. It has further been shown [7] that when present at high concentrations, the C_9 and C_{12} diacids are strong competitive inhibitors of mitochondrial oxidoreductases of the electron transport chain, whereas the C_6 diacid is only marginally so. These results can help to explain the present observations, as follows.

Cell death and an effect on proliferation produced by C_9 ions at 10^{-1} M can largely be attributed to inhibition of mitochondrial oxidoreductases, because C_6 ions which do not possess this property of inhibition to the same extent, do not, at the same concentration, cause cell death, and an initial depression of cell proliferation can be attributed to an osmolar or Na ion effect (see above). C_{12} ions at high concentration (5×10^{-2} M) likewise operate to inhibit mitochondrial oxidoreductases and hence have a depressing effect on cell proliferation, though less than they would have at 10^{-1} M, were it possible to get the diacid salt into solution at this concentration.

The morphologic observations are in general in line with those on cell counts. That the extensive damage to mitochondria with 10^{-1} M $C_9$2Na (and the lesser though observable damage with 10^{-1} M $C_6$2Na) is a specific and not an osmolar or high Na ion effect, is clear from the fact that there was no observable damage to these organelles of cells exposed to equiosmolar NaCl. The minimal damage caused by the $C_6$2Na fits in with its demonstrated lower level of inhibition of mitochondrial respiration [7], and with its lesser effect on proliferation as shown by the cell counts. The absence, up to 6 h, of observable damage to the cell membrane or to other intracytoplasmic membranes indicates that the mitochondrion is a prime target for dicarboxylic acids, and that their antimitochondrial activity is an important factor in producing their cytotoxic effect.

The C_{12} diacid and/or its breakdown products have also been located in the nucleus of normal melanocytes and murine malignant melanoma cells in culture by ultrastructural autoradiography [8,9]. This has led us to suggest that part of the cytotoxic activity may be due to a direct effect on some stage of the mitotic cycle, possibly on DNA synthesis. Leibl, Pehamberger, Konrad et al [13] have, in fact, recently reported that azelaic acid at $1–4 \times 10^{-2}$ M produces a significant decrease of DNA synthesis in cultured melanoma cells.

REFERENCES

1. Nazzaro-Porro M, Passi S: Effetto degli acidi dicarbossilici in alcune dermatosi iperpigmentarie. G Ital Dermatol 113:101–104, 1978
2. Nazzaro-Porro M, Passi S, Balus L, Breathnach AS: Effect of dicarboxylic acids on lentigo maligna. J Invest Dermatol 72:296–305, 1979
3. Nazzaro-Porro M, Passi S, Zina G, Bernengo A, Breathnach AS, Gallagher S: Effect of azelaic acid on human malignant melanoma. Lancet 2:1109–1111, 1980
4. Nazzaro-Porro M, Passi S, Zina G, Breathnach AS: Die Wirkung von Azelainsäure auf Hyperpigmentierungen. Hautarzt (Suppl 6) 34:220–221, 1983
5. Nazzaro-Porro M, Passi S, Zina G, Breathnach AS: Effects des substances mélanocytotoxiques sur les mélanocytes malins, Le Mélanome Malin: Rapports Presentés au XVIIe Congrès de L'Association des Dermatologistes et Syphiligraphes de Langue Francaise. Berlin, Schering AG, 1983, pp 197–214
6. Ertle T, Wiskemann A, Jänner M: Local treatment of lentigo maligna with azelaic acid. Arch Dermatol Res 271:197–203, 1981
7. Passi S, Picardo M, Nazzaro-Porro M, Breathnach AS, Confaloni AM, Serlupi-Crescenzi G: Anti-mitochondrial effect of saturated medium chain length (C_8–C_{13}) dicarboxylic acids. Biochem Pharmacol 33:103–108, 1984
8. Breathnach AS, Ward BJ, Robins EJ, Ethridge L, Nazzaro-Porro M, Passi S: Analytical ultrastructural autoradiographic localization of a dicarboxylic acid in cultured human epidermal cells (abstr). J Invest Dermatol 80:370, 1983
9. Ward BJ, Breathnach AS, Robins EJ, Bhasin YP, Ethridge L, Passi S, Nazzaro-Porro M: Analytical ultrastructural autoradiographic and biochemical studies on ^{3}H dicarboxylic acid added to cultures of melanoma cells. Br J Dermatol 111:29–36, 1984
10. Russell WC, Newman C, Williamson DH: A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature 253:461–462, 1975
11. Moroney MJ: Facts from Figures, 2nd edition. Harmondsworth, Penguin Books, 1954
12. Passi S, Nazzaro-Porro M, Picardo M, Mingrone G, Fasella P: Metabolism of straight, saturated, medium chain-length (C_9 to C_{12}) dicarboxylic acids. J Lipid Res 24:1140–1147, 1983
13. Leibl H, Pehamberger H, Konrad K, Stingl G, Wolff K: Effect of azelaic acid on melanoma cells (abstr). Arch Dermatol Res 276:273, 1984