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MODIFICATION OF DOPA TOXICITY IN HUMAN TUMOUR CELLS

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Abstract—A variety of factors were found to modify the toxicity of L-dopa in HeLa cells (D_{37} 16 μM) and in dopa-sensitive, nonpigmented human melanoma cells (MM96) (D_{37} 5 μM) having a similar size and doubling time. Dopa toxicity was decreased by concurrent treatment with superoxide dismutase, peroxidase or catalase, by erythrocytes, or by hypoxia. Toxicity could be increased by the enzyme inhibitors L- and D-penicillamine, sodium diethyldithiocarbamate or 3-amino-1,2,4-triazole. The two cell lines had similar levels of superoxide dismutase and peroxidase; in 6 human melanoma lines, no correlation was found between dopa killing and tyrosinase activity as determined either by formation of dopa from tyrosine or by formation of melanin from dopa. Uptake of L-dopa was similar in HeLa and MM96 cells, and the toxicity of D-dopa was the same in both lines as that of the L-isomer. Dopa decomposed within 12 hr in culture medium, the rate and products being influenced by addition of the above enzymes and by the cell density. Dopa-melanin and medium containing decomposed dopa were also selectively toxic to MM96 cells. Adenovirus 5 was used in two different ways to assess the relative importance of DNA damage and inhibition of DNA synthesis by dopa. Viral replication was found to be unaffected in cells being treated with dopa but was strongly inhibited in cells treated with the DNA polymerase inhibitor cytosine arabinoside. Secondly, the virus was itself inactivated by treatment with dopa for 24 hr (D_{37} 1.3 mM); similar dose response curves were obtained for replication of dopa-treated virus in untreated HeLa or MM96 cells. These results show that the initial events of dopa toxicity occur outside the cell and lead to the formation of a stable, toxic product (probably melanin) which does not strongly inhibit DNA polymerase activity. Melanoma hypersensitivity was not due to differences in oxygen-metabolizing enzymes, dopa uptake, or DNA repair.

Following the initial reports of DNA damage and *in vivo* activity against rodent tumours [1, 2], dopa and other catechols were shown to be active against mouse melanoma [3, 4] neuroblastoma [5] and leukaemia [6, 7] and to have selective toxicity towards mouse [4] and human [8–10] melanoma cells *in vitro*. Clinical trials of dopa therapy appear to be promising, the present limitation being cardiovascular toxicity [11].

The mechanism of catechol toxicity and its apparent selectivity *in vitro* for melanoma and other tumour [10] cells is not yet clear. The observed arrest of cells at the G1/S interface, and inhibition of DNA synthesis in cells and in a cell-free system may indicate a toxic effect similar to that of cytosine arabinoside [6, 12] and be due to inhibition of DNA polymerase [13]. Inhibition of ribonucleotide reductase has also been reported [14]. On the other hand, DNA degradation [2] and DNA breaks [8] have been detected. Melanisation *per se* did not appear to be a major determinant for human melanoma selectivity [8] but this does not exclude a possible role for one of the earlier control points [15] in the melanin biosynthetic pathway. Transport of [^{3}H]dopa was enhanced in suspensions of human melanoma cells compared with fibroblasts but not when cell monolayers were used [8]; because of the instability of dopa in solution, this test did not distinguish between dopa and dopa decomposition products.

The combination of ascorbate and copper ion is also selectively toxic for melanoma cells *in vitro* [8, 16, 17]. It therefore seemed feasible that redox systems with a suitable electron donor generate a species capable of inducing cell damage, melanoma selectivity arising either from enhanced transport of the electron donor or of an intermediate, or from an intracellular difference related perhaps to elements of the melanin biosynthetic pathway.

We have explored these possibilities by comparing the survival and relevant enzyme activities of HeLa cells with those of an amelanotic dopa-sensitive melanoma cell line having a similar size and doubling time. Secondly, the previous finding that high cell densities greatly decreased dopa toxicity [8] prompted a study of factors which might increase toxicity *in vivo*. Thirdly, the question of DNA damage, DNA repair or inhibition of DNA polymerase being involved in the mechanism was addressed by comparing the replication of the DNA virus adenovirus 5 in sensitive and resistant cells, a system where genome damage could be studied separately from enzyme damage using intact cells.

MATERIALS AND METHODS

The human melanoma line MM408 was established from a lymph node metastasis from a 31-year-old male. The origins of HeLa-S_3 and the other human melanoma cell lines have been described [8]. The melanotic subline of MM96 (MM96E) was obtained from early passage stocks and the amelanotic subline (MM96L) from late (>100) pass-

age. MM96L was used in all experiments unless otherwise stated.

Cells were cultivated at 37° in 5% CO_2/air in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Commonwealth Serum Laboratories, Melbourne, Australia) containing 100 μg streptomycin per ml, 100 IU penicillin per ml, 3 mM 4-2 (hydroxyethyl)-1-piperazine-ethanesulphonic acid, and 10% (v/v) foetal calf serum. Cell survival was determined by a colony-type assay [8], involving treatment of 2×10^3 cells/16 mm well and pulse labelling of colonies with [^{3}H]thymidine 6–7 days after treatment. Cells for enzyme studies were harvested in exponential phase, suspended in phosphate-buffered saline (5×10^7/ml), lysed with 0.2% Triton X-100 and stored at −10°.

Dopa was estimated by the method of Husain *et al.* [18], modified as follows. The sample to be assayed (100 μl) was mixed with 0.9 ml of 40 mM sodium phosphate − 1 mM zinc sulphate (pH 6.5) and 100 μl of 10 mM potassium ferricyanide. After 2 min, 100 μl of a mixture of 4.5 N NaOH and 10 mM ascorbic acid was added, diluted with 2.5 ml H_2O after a further 5 min, and the fluorescence read using a Turner model 111 fluorometer (excitation filter 7–37, emission filter 2-A with 10% neutral density filter). The L isomer was used in all experiments unless otherwise stated.

Superoxide dismutase activity was detected colorimetrically as inhibition of the reduction of nitro blue tetrazolium by photochemically-generated superoxide ion [19]. Potassium phosphate buffer (1 ml; 50 mM, pH 7.8) containing 56 μM nitro blue tetrazolium, 20 mM methionine, 1.2 μM riboflavin, 20 μm potassium cyanide, and 10 μl of cell lysate, was irradiated for 0–15 sec with 3 × 250 W Philips Photoflood lamps at a distance of 10 cm. The change in absorbance was read at 560 nm. Superoxide dismutase from bovine erythrocytes (3000 U/mg; Calbiochem, La Jolla, CA) was used as a positive control.

For assay of peroxidase, 1 ml of 0.1 M sodium phosphate buffer (pH 7.5) containg 0.3 mM *o*-dianisidine was mixed with 200 μl cell lysate and 100 μl 200 mM hydrogen peroxide. The increase in absorbance at 25° was followed at 440 nm. Horseradish peroxidase (Calbiochem) was used as a positive control.

Tyrosinase was determined using the method of Husain *et al.* [18]. Cell lysate (20 μl) was incubated at 37° with 1 ml 40 mM sodium phosphate buffer (pH 6.8) containing 0.1 mM tyrosine, 5 μm dopa and 0.75 mM ascorbic acid. At 30 min intervals, 100 μl aliquots were removed for estimation of dopa as described above.

The melanin content of cell lysates was calculated from the absorbance at 595 nm after complete lysis with 0.5% Sarkosyl. Synthetic melanin (Sigma Chemical Co., St Louis, MO) was used as the standard. Melanin formation in medium was determined similarly.

For determination of dopa uptake, cells seeded 24 hr previously (5×10^5 per 60-mm plate) were treated with dopa in complete medium (1 ml), washed rapidly (<10 sec) with phosphate-buffered saline pH 7.2 (2 × 5 ml) at 0° and the cells dislodged with 0.5 ml trypsin (0.2 mg/ml) in saline. After counting the number of cells (haemocytometer), the cell suspension was lysed with Triton X-100 (0.2%) and aliquots removed for determination of dopa as described above. A dish without cells was used as the blank. No correction was made for binding of extracellular dopa. Dopa uptake was calculated as pmole per cell and as the concentration ratio (concentration inside the cell ÷ concentration in the medium).

Adenovirus 5 was obtained from Dr A. Bellet, John Curtin School of Medical Research, Canberra. A stock of partially-purified virus was prepared by infecting 10^8 HeLa cells in a confluent monolayer with 50 ml of virus in medium (10^4 ID/ml).* After 3 days the cells were harvested, frozen and thawed 5 times in 20 ml medium and extracted with Arklone (2 × 10 ml) at 4°. The aqueous supernatant (7×10^6 ID/ml) was stored at −70°. No superoxide dismutase, catalase or peroxidase activity could be detected in this preparation. Virus replication was determined using a modification of the immunofluorescent method of Rainbow and Howes [20]. Infected cultures (5×10^3 cells/6-mm well) were cultured for 48 hr and then washed with phosphate-buffered saline, fixed with methanol for 1 min and incubated for 30 min at 37° with a 1/30 dilution of adenovirus 5 neutralizing antiserum (Microbiological Associates, Walkersville, MD) in phosphate-buffered saline (20 μl). After washing with buffer (3 × 0.2 ml), the cultures were incubated for 30 min at 37° with a 1/100 dilution of peroxidase-conjugated protein A (Kirkegaard and Perry Laboratories, Gaithersburg, MD) in phosphate-buffered saline (20 μl), washed twice with 2 mM Tris buffer (pH 7.4) and the cells containing replicating virus identified microscopically by brown nuclear staining after 5-min treatment at ambient temperature with a mixture of *o*-dianisidine (1 mM) and hydrogen peroxide (2.4 mM) in Tris buffer. One ID was defined as the minimum amount of virus required to produce one infected cell under the above conditions.

RESULTS

Cell survival

The survival curves in Fig. 1 confirmed the previous report [8] that MM96 cells were sensitive to killing by L-dopa compared with HeLa cells. Some variation in the absolute levels of survival were obtained in different experiments (Table 1), possibly due to small variations in the initial cell number [8] and in the catalase activity of foetal calf serum (see below). D-Dopa showed the same toxicity and selectivity for MM96 cells as the L isomer (Fig. 1); hydrogen peroxide, a possible product from aerobic oxidation of dopa, was equally toxic to both cell types. Synthetic melanin prepared by chemical oxidation of dopa was selectively toxic toward MM96 cells (Fig. 2A), and correlated with dopa toxicity in six cell lines (Fig. 2B). Compared on the basis of monomer concentration, dopa-melanin was approximately 20-fold less toxic than dopa itself (Table 1).

The toxicity of dopa was found to be highly sus-

* Abbreviations used: ID, infectious dose.

Table 1. Comparison of dopa toxicity, melanin toxicity, tyrosinase activity and melanin content in human tumour cell lines

Cell line	D_{37} for dopa (μM)*	D_{37} for melanin (μM)†	Tyrosinase (pmole/min/10^6 cells)	Melanin (pg/cell)
HeLa	20 ± 1.4 (7)‡	700	<0.5	<0.5
MM96L	4.4 ± 0.7 (8)	75	126	0.2
MM96E	6.0	—	1500	1.72
MM127	7.9 ± 0.3 (2)	125	<0.5	<0.05
MM138	0.75 ± 0.05 (2)	60	1.8	<0.05
MM253c1	18 ± 1 (2)	650	3.0	0.05
MM408	13 ± 1 (2)	272	240	2.4

* Dose required to reduce survival to 37%.
† Equivalent concentration of dopa monomer.
‡ Mean and S.E. for separate experiments. Number of experiments in parenthesis.

ceptible to modification by other agents. Catalase, peroxidase or superoxide dismutase greatly reduced dopa killing (Table 2); the effect was not additive when these enzymes were used together (results not shown). Inhibitors of these enzymes, on the other hand, increased dopa toxicity (Table 2). Diethyl dithiocarbamate and both isomers of penicillamine appeared to have some selectivity against MM96 cells when used alone, thus limiting the concentration which could be used with dopa.

The toxicity of dopa was reduced by incubating the cultures in a 5% oxygen atmosphere instead of in air, or by addition of human erythrocytes. No hemolysis was detected during the latter experiment.

Decomposition of dopa

The stability of dopa in culture medium was assayed chemically and by cell survival. The rate of formation of melanin was low during the first 5 hr but then accelerated, so that decomposition of dopa by this pathway appeared to be complete within 16 hr (Fig. 3A). A mirror image temporal response was obtained by following the loss of dopa (Fig. 3B). Peroxidase accelerated the decomposition of dopa to melanin; superoxide dismutase and catalase retarded dopa decomposition, but not at the expense of melanin formation (Fig. 3).

The presence of MM96 and HeLa cells at low density had little effect upon the rate of dopa decomposition in culture medium (Fig. 4). High cell densities, however, inhibited melanin formation and delayed, but did not prevent, the decomposition of dopa.

Cell survival studies showed that the toxicity of dopa was not greatly reduced by preincubation and consequent decomposition in culture medium except when HeLa cells were treated with dopa preincubated for 24 hr (Fig. 5). The effect of the duration of dopa treatment upon cell killing was also determined. At equitoxic doses little effect was found in HeLa cells unless treated for at least 24 hr whereas in MM96 cells, toxicity was detected after treatment for 8 hr (Fig. 5).

Uptake of dopa

Since the survival studies utilised cells attached to a plastic surface, dopa uptake studies were limited to the use of cell monolayers. The validity of the method was indicated by the fact that after 2 washes with cold buffer, 2 additional washes removed <10% of the remaining activity. The MM96 and HeLa lines both exhibited a rapid uptake of dopa and reached a plateau within 5 min (Fig. 6A), similar to the results

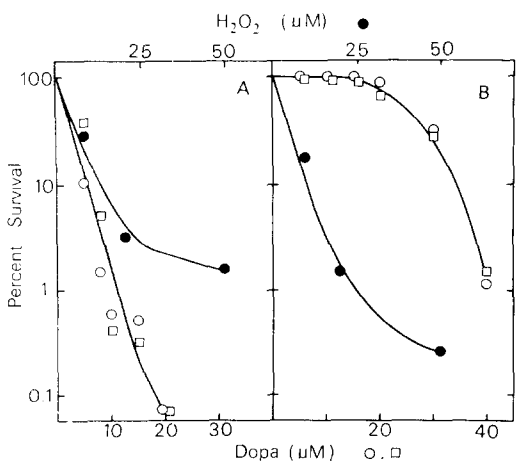

Fig. 1. Survival of MM96L and HeLa cells (A) MM96L: ○, L-dopa; □, D-dopa; ●, H_2O_2. (B) HeLa: same symbols as in A. The points are means of duplicates. All S.D. <10%.

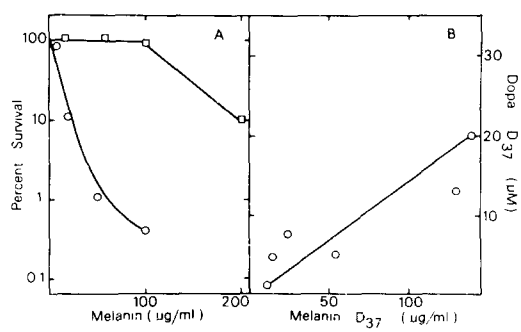

Fig. 2. Correlation of melanin and dopa toxicity. (A) Toxicity of melanin in MM96L (○) and HeLa cells (□). (B) Correlation of D_{37} values for melanin and L-dopa in six cell lines listed in Table 1.

Table 2. Modification of L-dopa toxicity by other agents

Agent*	Concentration	Modification factor† MM96	HeLa
Enzymes			
Catalase	900U/ml	0.14	0.18
Peroxidase	0.6U/ml	0.17	0.13
Superoxide dimutase	60U/ml	0.29	0.36
Inhibitors			
D-penicillamine	0.33 mM	3.4	5.3
L-penicillamine	0.33 mM	4.2	5.0
Diethyl dithiocarbamate	0.05 μM	2	1.7
3-amino,1,2,4-triazole	1 mM	3.0	4.0
Others			
Erythrocytes‡	5×10^7/ml	<0.1	<0.1
Low O_2	5%	0.36	0.50

* Added just before dopa (85–100% survival when used alone).
† D_{37} for dopa/D_{37} for the combination.
‡ Human erythrocytes from a normal donor, washed in phosphate-buffered saline and stored at 4°.

obtained previously using isotopically-labelled dopa [8]. The level of the plateau responses was linearly-dependent upon the external dopa concentration in the range 10–1000 μM (Fig. 6B).

Cellular enzyme activities

Because of the catalase activity in these cells [8], a level of hydrogen peroxide 10-fold higher than normal was required for assay of peroxidase. Both cell lines had similar levels of peroxidase and superoxide dismutase activities (Table 3). Tyrosinase, assayed by following the conversion of tyrosine to dopa under conditions which prevent further oxidation to melanin [18], was not detectable in HeLa cells but a high activity was found in MM96 cells.

The complexities of melanin biosynthesis prompted the use of assays applicable to different stages in the pathway. Lysates of HeLa cells strongly inhibited the spontaneous oxidation of dopa to

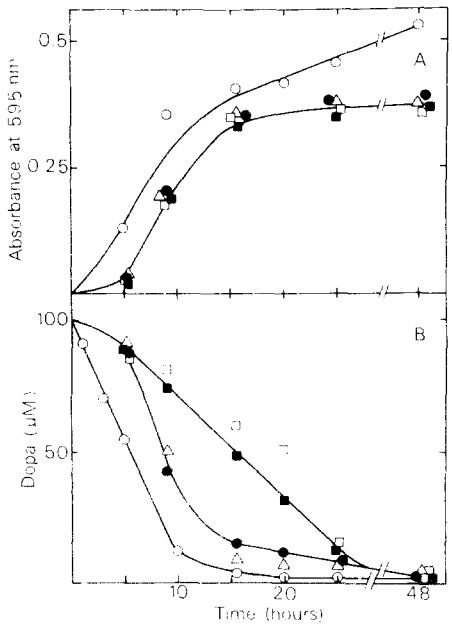

Fig. 3. Rate of decomposition of 100 μM L-dopa in complete medium at 37°. (A) Formation of melanin: △, control; ○, 0.6 U/ml peroxidase; □, 60 U/ml superoxide dismutase; ■, 900 U/ml catalase; ●, 0.33 mM D-penicillamine. (B) Loss of dopa. Same symbols as in A. The points are means of triplicates. All S.D. <10%.

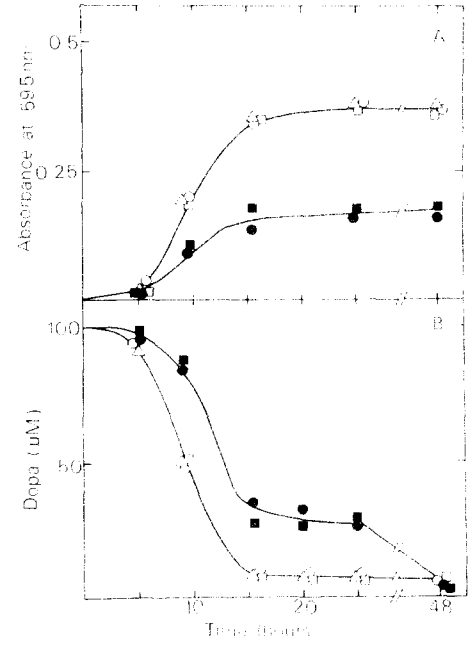

Fig. 4. Effect of cell density on the decomposition of 100 μM L-dopa in medium at 37°. (A) Formation of melanin: △, control (no cells); ○, 2×10^5 MM96L cells per 60-mm dish; □, 2×10^5 HeLa cells; ●, 2×10^5 MM96L cells; 2×10^5 HeLa cells. (B) Loss of dopa: same symbols as in A. The points are means of triplicates. All S.D. <10%.

Table 3. Comparison of enzyme activities in lysates of HeLa and MM96 cells

Enzyme	Activity (pmole/min/10^6 cells)		
	No cells	HeLa	MM96
Peroxidase	10	42	33
Superoxide dismutase*	<0.1	1.1	1.3
Tyrosinase			
(a) formation of dopa	<0.5	<0.5	126
(a) loss of dopa	150	19	940
(b) formation of melanin	26	<0.1	53

* Units of activity per 10^6 cells. One unit is defined as 50% inhibition of the reduction of nitro blue tetrazolium by superoxide ion.

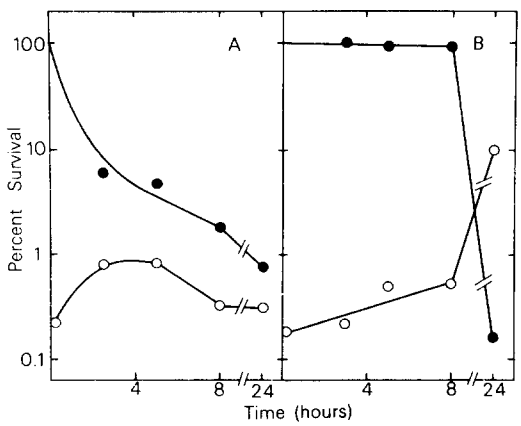

Fig. 5. Effects of duration of dopa treatment and dopa preincubation on cell survival. For varying the duration of dopa treatment, cultures set up for the standard cell survival assay were treated with dopa and washed twice with fresh medium at various times afterwards. In the dopa preincubation experiment, dopa was incubated in medium for various times, added to untreated cultures and allowed to remain on the cells for the duration of the experiment. (A) MM96L cells treated with 10 μM dopa: ●, dopa treatment time; ○, dopa preincubated at various times before being used for continuous cell treatment. (B) HeLa cells treated with 40 μM dopa: same symbols as in A. The points are means of duplicates. All S.D. <10%.

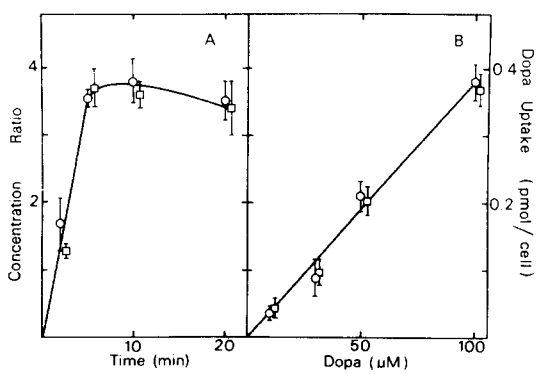

Fig. 6. Temporal and concentration dependence of L-dopa uptake by tumour cells. (A) Temporal dependence of uptake of 50 μM L-dopa by MM96 (○) and HeLa cells (□). (B) Concentration dependence of dopa uptake by MM96L (○) and HeLa cells (□), incubated for 10 min. The points are means and S.D. of triplicates.

melanin (Table 3) whereas MM96 cells had the opposite effect; assay of dopa loss or melanin formation gave qualitatively similar results.

The possibility that dopa sensitivity was correlated with tyrosinase or melanin content was assessed by comparison of 7 cell lines, including the autologous pair MM96L and MM96E. These lines varied widely with respect to the above properties, but no correlation was found with the D_{37} for dopa (Table 1).

Adenovirus assays

Replication of adenovirus in tumour cell lines was conveniently measured using an immunoperoxidase modification of the viral antigen assay described by Rainbow and Howes [20]. The method gave results similar to those obtained using plaque or end-point dilution assays (not shown). Scoring of peroxidase-labelled cells (0–2% of cells in each well) was carried out in wells near the end-point (10^{-5}–10^{-6} virus dilution when no drug was used). The number of ID per cell was therefore always less than 1. In the wells

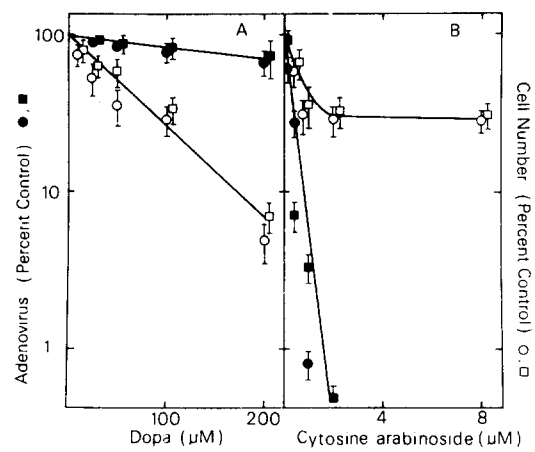

Fig. 7. Replication of adenovirus 5 in drug-treated cells. Cultures were incubated with virus dilutions of 10^{-2}–10^{-6} for 60 min and then washed. Drugs were added after 8 hr and after a further 48 hr the virus titre was determined as described in the text. The number of cells remaining in wells at the virus end point was also counted. (A) L-Dopa; ●, replication in MM96L; ■, replication in HeLa; ○, MM96 cell number; □, HeLa cell number. (B) Cytosine arabinoside: same symbols as in A. The points are means and S.E. of 3 separate experiments.

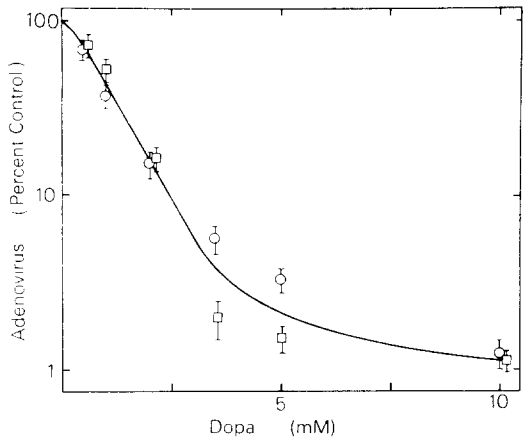

Fig. 8. Replication of L-dopa-treated adenovirus 5 in tumour cells. A 10^{-3} dilution of virus was treated with dopa in complete medium (5 ml) in a 60-mm plate at 37° for 24 hr. Cells were infected with virus for 30 min and washed twice with medium. After 48 hr in culture the virus titre was determined as described in the text: ○, MM96L cells; □, HeLa cells. The points are means and S.E. of 3 separate experiments.

infected with lower viral dilution, the cytopathic effects of viral replication were readily apparent as cell detachment and lysis.

In the first set of experiments, the ability of adenovirus to replicate in drug-treated cells was tested. Since viral DNA synthesis occurrs 8–10 hr after infection [21] when inhibition of cellular DNA synthesis by dopa may have subsided [8], the drug treatments were applied 8 hr after infection. Under these conditions, and using supratoxic dopa levels up to the point of causing heavy cell loss after 2 days, viral replication was barely affected (Fig. 7A). Similar results were obtained when dopa treatment was commenced 1 hr after infection. Production of viral antigen was not due to an abortive infection because the usual cytopathic effects were obtained at lower virus dilutions, and infectious virus was demonstrated in these wells by transferring the medium to control cultures. Cytosine arabinoside (D_{37} 0.2–0.4 μM for HeLa and MM96) was less toxic to the cells but strongly inhibited viral replication (Fig. 7B).

The second set of assays showed that adenovirus could be directly damaged by dopa treatment. Incubation of virus with dopa in complete medium for 24 hr gave a viral D_{37} of 1.2 mM in both MM96L and HeLa cells (Fig. 8). The 10–100 fold higher level of dopa required to inactivate virus compared with cells is similar to that reported for other DNA-damaging agents such as u.v. and ionizing radiation [20, 22], presumably due to the smaller target size of the viral genome. As a control to show that dopa composition products present during the 30 min virus infection period did not affect the cells, separate cultures were treated for 30 min with preincubated L-dopa and then infected with control virus.

DISCUSSION

The similar toxicity of L- and D-dopa, the equal rates of dopa uptake by the sensitive and resistant lines and the strong protective effect of catalase and superoxide dismutase added to the culture medium indicate that initial stages in the mechanism of dopa killing take place outside the cell. These results are consistent with the possibility that aerobic oxidation of extracellular dopa leads to generation of superoxide ion which then not only decomposes to hydrogen peroxide but in conjunction with this product forms toxic hydroxyl radicals *via* the catalysed Haber-Weiss reaction [23]. This hypothesis also explains the lack of correlation of dopa sensitivity with hydrogen peroxide sensitivity. Peroxidase accelerated the spontaneous decomposition of dopa but since the decomposition products were also toxic the protective effect of this enzyme might be best explained by its removal of hydrogen peroxide. The synergistic effect of the enzyme inhibitors on dopa toxicity reinforces the above proposal and the equal effects of D- and L-penicillamine, only the latter isomer being taken up by cells [24], strongly suggest that this stage of the toxicity mechanism takes place outside the cell. It seems unlikely that penicillamine and diethyldithiocarbamate act merely as chelators of free copper ion because added copper did not inhibit dopa killing; however, other roles for these compounds cannot yet be completely excluded.

The coincident toxicity and selectivity of dopa, solutions of decomposed dopa and dopa-melanin suggest that melanin, the stable end-product of dopa oxidation, is an important mediator for *in vitro* killing of human cells by dopa. This finding contrasts with the dopa-sensitive Cloudman S-9 mouse melanoma which was resistant to dopa-melanin [25]. In the present work, superoxide dismutase and catalase inhibited dopa toxicity without preventing the formation of melanin. Thus melanin, which is known to produce radicals during synthesis or on irradiation [26, 27], may initiate its toxic effect outside of the cell.

No evidence was obtained to identify the link between the putative hydroxyl radicals formed outside the cell and the DNA breaks and inhibition of DNA synthesis previously associated with dopa sensitivity [4, 7, 8]. The similar toxicity of dopa in complete medium and in phosphate buffer excludes the possibility of toxic products being generated from organic components in the medium. Dopa may be converted to an optically-inactive quinone such as melanochrome or related compounds [25] capable of causing damage upon entering the cell, but cells with high tyrosinase levels would then be expected to be the most sensitive because of intracellular synthesis of melanochrome from dopa. Another explanation is that the initial damage occurs to the cell membrane, DNA breaks [8] and inhibition of DNA synthesis [4, 7, 8] being secondary effects which eventually result in cell death.

The proliferation of adenovirus in cells treated with toxic levels of dopa showed that enzymes, including DNA polymerase, were still sufficiently active for replication of the 2×10^7 dalton viral genome. Inhibition of cellular DNA synthesis by dopa in intact cells may therefore be due to template damage as proposed for u.v. [28], to a low level of polymerase inhibition unrelated to dopa toxicity, or to effects on replication processes peculiar to the

mammalian genome. Because different target cells were used, these conclusions may not apply to the systems studied in other laboratories [6, 9–14, 25, 29–31].

The reason for melanoma hypersensitivity, and for some, but not all, human melanoma cell lines being highly susceptible to dopa, is not yet clear. Host cell reactivation of DNA viruses is a general method for detecting (but not identifying) differences in cellular repair of damaged DNA [20, 22]. The present results with adenovirus therefore indicate that if dopa killing results primarily from DNA damage, melanoma hypersensitivity is unlikely to be due to a deficiency in DNA repair. The similar effect on dopa-sensitive and -resistant lines of the various modifying agents indicate that at least the early stages of dopa killing were the same in the opposing cell types. Unless specially compartmented, differences in catalase [8] and 3 other enzyme activities related to oxygen metabolism appeared to have no correlation with dopa killing. Other avenues of investigation remain, however, such as the metalloproteins required to catalyse the Haber-Weiss reaction [23] and the possible role of factors in the melanin biosynthetic pathway in addition to those reported [15]. Concerning the latter, it was of interest that HeLa cell lysate inhibited the spontaneous conversion of dopa to melanin, suggesting that in a melanoma cell the levels of differentiation-independent enzymes such as catalase and superoxide dismutase could influence melanisation.

The lowering of dopa toxicity by physiological-level oxygen tension and by human erythrocytes (one hundredth physiological level) supports the previous suggestion [8] that the *in vitro* mechanism of dopa killing is likely to be strongly suppressed *in vivo*. However, the results of this study also raise the possibility that certain inhibitors such as penicillamine, already used to treat various disorders in humans [24], may enhance an oxidative and perhaps antitumour pathway of catechol metabolism *in vivo*.

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