

## THE EFFECTS OF DEPIGMENTING AGENTS ON THE GROWTH OF A TRANSPLANTABLE HAMSTER MELANOMA

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**Abstract.** The monoethyl ether of hydroquinone, 4-methyl catechol, *N* (2-mercaptoethyl) dimethylamine HCl, and parateritary butyl phenol are known to destroy selectively normal melanocytes in vivo. These compounds were injected into golden hamsters bearing Fortner's melanotic melanoma no. 1. No depigmentation or tumor growth inhibition was observed and host survival time was not prolonged.

Depigmenting agents have been the subject of study for over 30 years. Clinical observation and experimental testing have increased the known number of depigmenting agents and have shown that the depigmentation is the result of melanocytic degradation and disappearance. These clinical and experimental findings are reviewed briefly.

In 1936 Oettel (9), during study of food preservatives, fed hydroquinone to black cats and observed that the cats became gray. Three years later, Oliver et al. (10) investigated the occurrence of leukoderma in tannery workers and found that the leukoderma was caused by the monobenzyl ether of hydroquinone, an antioxidant contained in the workers' rubber gloves. The authors found that this compound can produce depigmentation of human skin if applied daily by patch test for 2 to 4 weeks. Dopa staining of biopsy material showed an absence of dopa-positive particles in the basal layer of the leukodermic area.

In 1966 Chavin & Schlesinger (3) described depigmentation in black goldfish after intradermal injection of 2-mercapto-ethylamine HCl (MEA) and *N* (2-mercaptoethyl) dimethylamine HCl (MEDA). Frenk et al. (6) applied these agents

topically to the epilated skin of black guinea pigs and found by dopa reaction a selective reduction in the number of melanocytes and degradation of those remaining.

Bleehen et al. (1) screened a series of thiols, catechols and quinones for depigmentary activity when applied topically to epilated black guinea pigs. The most effective compounds were 4-isopropyl catechol (4-ICP) and 4-methyl catechol (4-MC). Dopa staining of biopsies from 4-ICP treated areas showed marked reduction of the melanocyte population and degeneration of remaining melanocytes. Keratinocytes were unaffected.

Kahn (7) studied depigmentation which occurred in hospital personnel working with disinfectants containing phenolic compounds. He found on patch testing that each of the phenolic compounds could cause depigmentation of human skin. Biopsies were taken from depigmented sites. Dopa staining showed very sparse and enlarged melanocytes. Parateritary butyl phenol (ptBP), parateritary amyl phenol, and orthobenzyl, parachlorophenol, were the most effective depigmenting agents. PtBP was the least irritating. Earlier, Russian and Japanese authors also reported human and laboratory animal depigmentation after exposure to ptBP (8).

The above work indicated that certain hydroquinones, mercaptoethylamines, catechols and para-substituted phenols can cause selective destruction of normal melanocytes.

This study was undertaken to determine whether the more active compounds from each of these groups had any destructive or inhibiting activity on melanoma melanocytes.

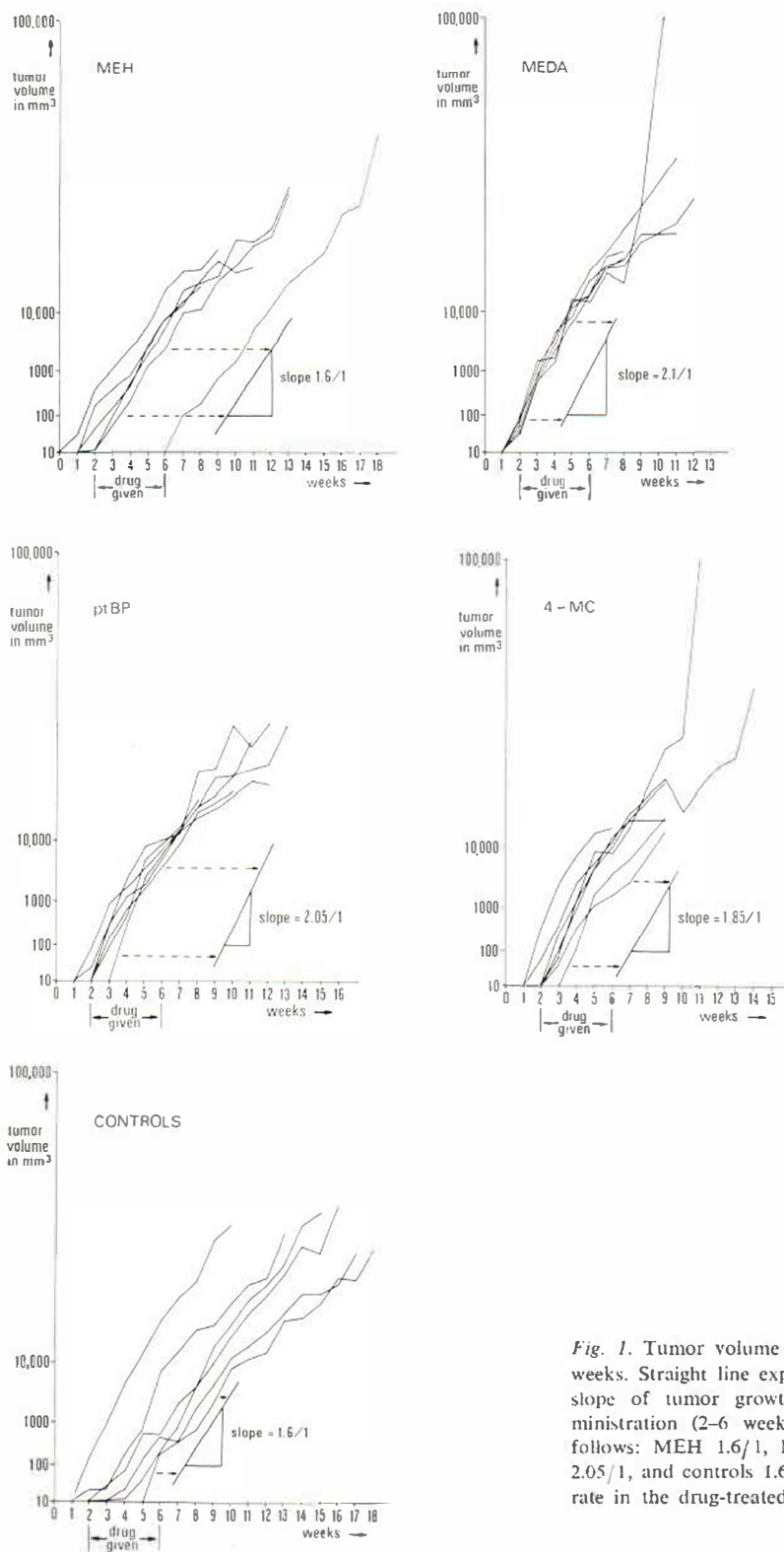


Fig. 1. Tumor volume in mm<sup>3</sup> is plotted against time in weeks. Straight line exponential growth is obtained. Mean slope of tumor growth rate during time of drug administration (2-6 weeks) is shown. Mean slopes are as follows: MEH 1.6/1, MEDA 2.1/1, 4-MC 1.85/1, ptBP 2.05/1, and controls 1.6/1. No inhibition of tumor growth rate in the drug-treated groups occurred.

## MATERIALS AND METHODS

Melanotic melanoma no. 1 of Fortner was maintained in a colony of golden hamsters by trocar subcutaneous implantations biweekly. Adult hamsters from 2 to 6 months of age were used. In comparing relative depigmenting activity of compounds within the hydroquinones, mercaptoethylamines, catechols and para-substituted phenols considerable weight was given to the reports of Brun (2), Chavin (4), and Blechan (1). Monoethyl ether of hydroquinone (MEH), *N* (2-mercaptoethyl) dimethylamine HCl (MEDA), 4-methyl catechol (4-MC) and parateritary butyl phenol (ptBP) were selected for study.

Toxicity studies were done to determine dosage and vehicles for subcutaneous injection. Both MEDA and 4-MC are soluble in water. Doses of 0.01 moles MEDA in 1 ml of water and 0.01 moles 4-MC in 1 ml of water were given. MEH and ptBP are insoluble in water. The following vehicles and combinations of vehicles were tested for solubility and for toxicity: water, water with 1% Tween 80, ethanol with and without alkylation with NaOH, acetone, DMSO, propylene glycol, peanut oil, *N*-methylformamide and *N*-methylacetamide. A solvent of 50% propylene glycol, 20% *N*-methylacetamide and 30% water was selected. Doses of 0.1 moles MEH in 0.1 ml of this solvent and 0.1 moles ptBP in 0.1 ml of this same solvent were given. All doses were administered subcutaneously at rotating sites once daily, 5 days a week for 4 weeks. Injections were begun 2 weeks after trocar implantation of 3 mm<sup>3</sup> chunks of tumor tissue. An initial tumor growth phase of 2 weeks was allowed, because at 2 weeks most animals bore visible and palpable tumors. The tumor size at 2 weeks is felt to correspond to the time of clinical diagnosis and treatment of human malignant melanoma.

Length, breadth and depth of each tumor was measured weekly with calipers using a millimeter scale. Volumes were calculated as ellipsoids,

$$V = \frac{4\pi}{3} \frac{abc}{6}$$

Weekly tumor growth rates were obtained. Volume of water displacement provided a measure of tumor size at death of the animal. Animal survival times and incidence and location of metastases were also studied.

## RESULTS

A total of 33 animals were studied. This number excluded 9 animals whose deaths were clearly traumatic and caused by other animals in common cages. The 33 animals were drawn from 5 groups. 7 animals comprised the control group; 6 received MEH; 7 received MEDA; 7 received 4-MC; 6 received ptBP. The total number included 8 animals whose primary tumors had ulcerated. The data do not indicate that ulceration shortened mean survival time. However, the tumors of these 8 animals were excluded neces-

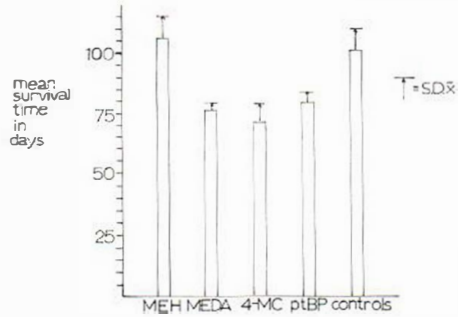


Fig. 2. Mean survival time for each group of animals is shown in days. Mean standard deviation (S.D.  $\bar{x}$ ) is given. Animals which received MEDA, 4-MC, and ptBP died sooner than animals in the control and MEH groups.

sarily from measurement of tumor mass at time of death.

To visualize tumor growth rate during the period of drug administration (2 to 6 weeks after tumor implantation), the data were plotted on semilog paper, giving straight lines (exponential growth). Mean slopes were obtained on visual inspection. No inhibition of tumor growth rate in the drug-treated groups could be seen (Fig. 1).

Animal survival times (Fig. 2) and tumor volumes at death (Fig. 3) were recorded. Animals which received MEDA, 4-MC, and ptBP died sooner than animals in the control and MEH groups. Yet the 5 groups of animals showed no significant difference in mean tumor volumes at death. The shortened survival time of the MEDA, 4-MC and ptBP treated groups is reflected in the slightly steeper slopes of tumor growth rate (Fig. 1). There is no clear explanation for this. MEDA, 4-MC and ptBP are not known to cause tumor growth enhancement or immunologic impairment, and no difference in the number or

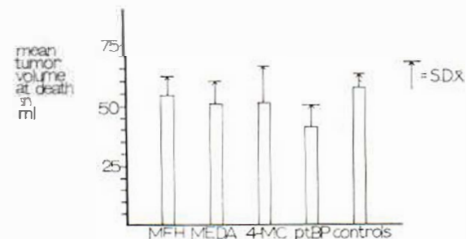


Fig. 3. Mean tumor volume at death for each group of animals is shown in milliliters. Mean standard deviation (S.D.  $\bar{x}$ ) is given. The groups of animals showed no significant difference in mean tumor volumes at death.

size of metastases is seen. All animals showed pulmonary metastases and many had metastases to mediastinum (48%), heart (30%), mesenteric nodes (27%), kidneys (24%), right axillary nodes (24%), and flank and para-aortic nodes (15%). No diminution of either number or size of metastases occurred in the drug-treated groups.

## DISCUSSION

Riley (11, 12) has shown that the monomethyl ether of hydroquinone (MMH) is selectively incorporated into guinea pig melanocytes in cell cultures and that a new electron resonance spin signal is generated *in vivo* when MMH is applied topically to black guinea pig skin. He found that the amount of uptake is dependent upon the degree of tyrosinase activity and the state of pigmentation of the cells. Heavily pigmented cells exposed to MMH undergo cytoplasmic blebbing and cell membrane rupture. A similar effect was seen for catechols and para-substituted phenols. He suggested that these depigmenting agents undergo tyrosinase oxidation to form free radicals which diffuse from the melanosomes and initiate lipid peroxidation leading to cell damage. Riley's studies were done with normal melanocytes.

This study tests the effect of depigmenting agents on melanoma cells *in vivo*. Under these conditions cell damage and hence tumor growth inhibition are not seen. No decrement or change occurred in tumor growth rate, in animal survival time, in tumor mass at death of the animal or in the pattern of metastases in the experimental animals compared with that in controls.

The report of Frenk & Ott (5) on the effect of MEH on pigment cells appeared while this study was in progress. The present data confirm their finding of no inhibitory effect of MEH on Fortner's melanoma no. 1. This finding is extended in this report to other potent depigmenting agents. Frenk & Ott have pointed out the structural differences between melanocytes which are damaged by MEH and those which are not. They also suggested that a powerful glutathione system may protect melanoma cells from damage by MEH by reduction of the oxidized depigmenting compound. Other mechanisms such as rapid *in vivo* clearance

or degradation of the injected compounds, the altered membrane characteristics of melanoma cells, and possible increased concentration of tyrosinase inhibitors in melanoma cells may explain this lack of inhibition on tumor growth. From these animal studies, it seems unlikely that depigmenting agents will find use in the treatment of malignant melanoma.

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