Antioxidants and Cancer. IV. Initiating Activity of Malonaldehyde as a Carcinogen [1]

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SUMMARY—Malonaldehyde was applied once to the shaved backs of mice. After daily treatment with 0.1% croton oil, 52% of the mice had tumors at 30 weeks. In the same experiment, other mice were treated once with β-propiolactone, glycidaldehyde, or 7,12-dimethylbenz-[a]anthracene (DMBA), and then daily with croton oil. These animals had 44, 40, and 95% tumors, respectively, at 30 weeks. Twelve mg malonaldehyde applied daily proved toxic, sometimes fatally so. Five animals also had carcinomas of their internal organs. After daily treatment with 0.36 mg malonaldehyde, no animals died of carcinoma. The predicted reactivity of malonaldehyde was confirmed; after 1 hour, only 1.9% of the applied malonaldehyde was detectable. All skin treated with DMBA, benzo[a]pyrene, and 3-methylcholanthrene had increased malonaldehyde levels.—J Natl Cancer Inst 53: 1771–1773, 1974.

SEVERAL ANTIOXIDANTS have reduced tumor incidence in animals treated in different ways with various organ-specific carcinogens.

Selenium has been effective against 7,12-dimethylbenz[*a*]anthracene (DMBA)-croton oil-induced skin tumors (*1–4*) and against *N*-2-fluorenylacetamide (FAA)-induced liver and mammary tumors (*5–7*). Vitamin E reduced the number of DMBA-croton oil-induced skin tumors (*1, 3, 4*) and the number of fibrosarcomas induced by 3-methylcholanthrene (*8, 9*). Ascorbic acid reduced DMBA-croton oil-induced skin tumors (*1, 4*), reduced uroepithelial carcinomas (*10*), and inhibited growth of sarcoma 180 (*11*).

The artificial food additive antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and ethoxyquin inhibited the carcinogenicity of benzo[*a*]pyrene (BP) or 7,12-dimethylbenz[*a*]-anthracene (DMBA) on the forestomach of the mouse (*12*). BHA inhibited pulmonary adenoma of the mouse induced by DMBA, BP, urethan, or uracil mustard (*13*). BHT inhibited hepatomas and mammary tumors induced by N-OH-FAA and liver tumors induced by FAA in male rats (*14*). Other antioxidants such as 4-methyl-2,6-di-*tert*-butyl-phenol inhibited hepatic tumor formation in rats fed *p*-dimethylaminoazobenzene (*15*), and tetraethylthiuram disulfide reduced the number of forestomach tumors induced by benzo[*a*]pyrene (*16*).

Although not every antioxidant experiment was positive against carcinogenesis (*17*), the large number of positive effects (*1–16*) may indicate some general effect of antioxidants against carcinogenesis. Malonaldehyde, a product of peroxidative fat metabolism, is formed in animal tissues when their diet is deficient in antioxidants. The structure of malonaldehyde (I) resembles that of two isomeric compounds, glycidaldehyde (II) and β-propiolactone (III), previously shown to be carcinogenic (*18, 19*). Our objective was to see if malonaldehyde is also a carcinogen.

MATERIALS AND METHODS

Groups of 30 Swiss female 55-day-old mice were initiated once on shaved backs with 0.25 ml acetone containing 12 mg or 6 mg malonaldehyde, 0.3 mg propionaldehyde, 2.5 mg glycidaldehyde, or 0.125 mg DMBA. Controls included initiator groups for DMBA and malonaldehyde and groups given croton oil only, acetone only, and no treatment. After 3 weeks, the backs of the mice were treated daily 5 days a week for 30 weeks with 0.25 ml of 0.1% croton oil in acetone. Tumor incidence was noted each week. In another experiment, 30 mice were treated with 12 mg malonaldehyde daily for 9 weeks. After this proved toxic, the 0.36 mg malonaldehyde was applied daily for 39 weeks. Another group of mice was treated daily with 0.36 mg malonaldehyde for 48 weeks. All experimental animals were housed in metal cages and given Purina rat pellet diet and water ad libitum.

β-Propiolactone was purchased from Sigma Chemical Co., St. Louis, Missouri, and DMBA was obtained from Eastman Kodak Co., Rochester, New York. Malonaldehyde was prepared by shaking malonaldehyde *bis*-(dimethylacetal) with Dowex 50 (*20*). The product was stored at −20° C in acetone in the dark. The malonaldehyde *bis*-(dimethylacetal) and glycidaldehyde were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin.

The structure of malonaldehyde indicates that it should be very reactive and would be quickly oxidized to malonic acid. In a recovery study, 12 mg malonaldehyde was applied to the backs of twenty 55-day-old Swiss albino female mice. Groups of 4 mice were killed at 1, 2, 4, 8, or 24 hours after application. One g mouse skin in 10 ml 0.9% NaCl in water was homogenized for 3 minutes with a Tekmar tissue homogenizer Model SDT with a 100 EN shaft. Malonaldehyde levels were determined (*21*).

Malonaldehyde levels in mouse skin were also determined after several carcinogens dissolved in acetone were applied to 55-day-old Swiss albino female mice. Mice without treatment were controls. The compounds tested were 0.01% 3-methylcholanthrene (MCA), 0.01% BP, and 0.01% DMBA. All test compounds were dissolved in 0.25 ml acetone and applied once. Six animals were killed in each group on days 1, 5, 10, 15, and 20 after application.

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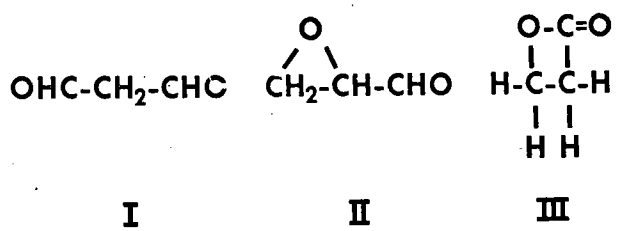


TEXT-FIGURE 1.—Chemical structure of malonaldehyde (I)' glycidaldehyde (II), and β-propiolactone (III).

Applications of the chemicals were timed so that all animals were killed on the same day.

RESULTS

The first tumors arose at week 5 in the mice treated with DMBA-croton oil, at week 11 in those with malonaldehyde-croton oil, and at week 16 in those with both β-propiolactone-croton oil and glycidaldehyde-croton oil (text-fig. 1). At 30 weeks, when the experiment was concluded, tumors appeared in 95% of the animals treated with DMBA-croton oil, 52% of both groups treated with 12 and 6 mg malonaldehyde-croton oil, 44% of the animals treated with β-propiolactone-croton oil, and 40% of those treated with glycidaldehyde-croton oil. Tumors were identified histologically. The skin tumors were keratoacanthomas. The backs of the malonaldehyde-treated animals turned orange within 10 minutes. No gross inflammation or skin damage was observed after malonaldehyde treatment. Of the mice treated with 12 mg daily, 12 died in the 4- to 6-week interval. In the next 3 weeks, 6 more died. Mortality and tumor incidence are summarized in table 1. Four animals had liver carcinoma; one, both kidney and rectum metastases; one, lung metastases; one, kidney metastases only; one, rectal carcinoma only; and one, no carcinoma.

When the concentration of malonaldehyde was decreased from 12 mg to 0.36 mg daily, no further tumors were observed through the 48th week. In the group treated with 0.36 mg was 1 keratoacanthoma, which appeared in the 43d week. The higher tumor incidence at 12 mg but not at 0.36 mg daily may relate to the threshold levels. Dietary antioxidants may prevent carcinogenesis by malonaldehyde at lower levels.

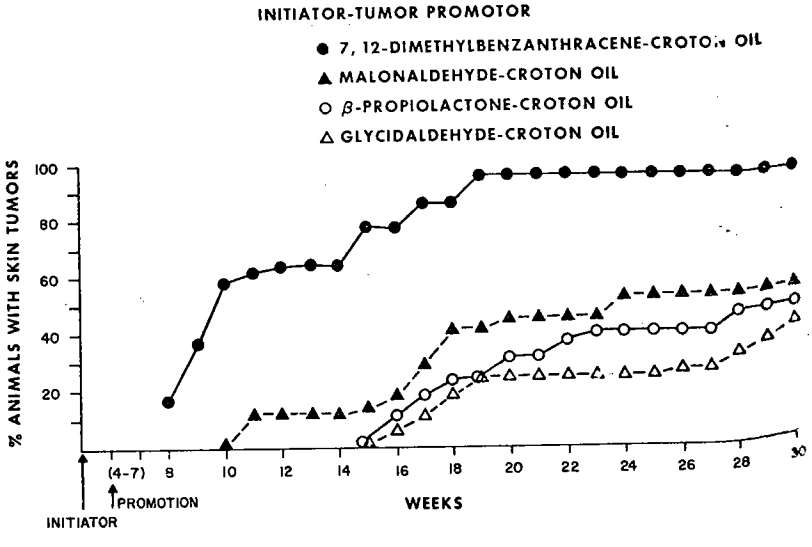
After 1 hour, 3220 nmoles malonaldehyde remained per g mouse skin. This was 0.23 mg, or about 1.9% of the 12 mg malonaldehyde originally applied. After 24 hours, 222 nmoles were still measurable (0.12%).

All carcinogens caused an increase of malonaldehyde within a few days of application. After application of benzo[*a*]pyrene, malonaldehyde continued to increase through day 20. Both MCA and DMBA increased malonaldehyde until day 10, when malonaldehyde declined in both groups. The increase of malonaldehyde after DMBA administration was similar to that previously reported (*4*).

DISCUSSION

The carcinogenicity of malonaldehyde, the structure of which closely resembles two known carcinogens, was not unexpected. There is much evidence that malonaldehyde might be the ultimate carcinogen. The known production of malonaldehyde in tissue of animals on low antioxidant diets is consistent with reports that antioxidants prevent carcinogenesis (*1–16*); all carcinogens tested produced malonaldehyde after application to mouse skin. Irradiation, another producer of carcinogenesis, also increases malonaldehyde levels (*22*). Dietary antioxidants prevented the formation of ultraviolet light-induced squamous carcinomas in the skin of hairless mice (*23*).

The expected reactivity of malonaldehyde is substantiated by the fact that only 2% of the substance remains after 1 hour. The backs of the mice turn a bright orange color within ten minutes after malonaldehyde application. The color probably reflects the Schiff's base formation when malonaldehyde reacts with protein (*24*). Malonaldehyde also reacts with DNA in vivo and in vitro (*20*). The increase of absorption at 325 nmoles may reflect production of a reaction product between malonaldehyde and adenine or guanine (*25*).



TEXT-FIGURE 2.—The effect of one application of DMBA, malonaldehyde, β-propiolactone, or glycidaldehyde on 30 mice. After 3 weeks, 0.25 ml of 0.1% croton oil was applied daily to each animal. Percentages of animals with tumors/week are indicated.

TABLE 1.—*Incidence of tumors other than skin tumors after application of malonaldehyde in 0.25 ml acetone to mouse skin*

Time (wk)	Malonaldehyde (mg/day)	Treatment group		Control group	
		Number alive	Tumors/dead animals	Number alive	Tumors/dead animals
4–6	12	18	0/12	30	0/0
7–9	12	12	5/6	30	0/0
10–48	0.36	12	0/0	28	0/2

The reports that antioxidants prevent carcinogenesis in animals (*1–16*) are also consistent with epidemiologic evidence. There is an inverse relationship between selenium occurrence and human cancer mortality in Canada (*26, 27*), New Zealand (*27*), and the United States (*27, 28*). Blood selenium levels are significantly lower in several types of cancer patients (*29*). A relationship has been postulated (*30*) between the declining American death rate from gastric carcinoma and the public acceptance of cereals rich in vitamin E and selenium and the introduction of food preservative antioxidants in 1947. The antioxidants *dl*-α-tocopherol, ascorbic acid, BHT, sodium selenite (*31*), and thiuram disulfide (*32*) have reduced DMBA-induced chromosome breakage.

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