

# Features of Tumor Enhancement by Croton Oil<sup>1</sup>

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## SUMMARY

Because of the possibility of cocarcinogenic effects of substances to which many people are exposed, the mechanism of action of one such agent was examined in 2-stage tumor induction experiments. The appearance of new tumors was studied at the end of a series of croton oil treatments, or between treatments given at long intervals in mouse epidermis given a single pretreatment with a carcinogen. During a series of treatments with croton oil, the tumor-enhancing effect of any single treatment persisted for 2 to 3 weeks. Tumors were enhanced by as few as 2 croton oil treatments when given 3 weeks apart, and it was also demonstrated that the concentration of croton oil was more important in tumor enhancement than the absolute amount.

## INTRODUCTION

Croton oil and some of its fractions (14) are capable of enhancing tumor growth in mouse epidermis previously exposed to an otherwise ineffective dose of a carcinogen. The use by civilized populations of agents with similar properties (e.g., detergents, benzene series solvents, etc.) is becoming increasingly common; their mechanism of action in tumor enhancement is, therefore, of interest. Additional features of tumor enhancement by croton oil are examined in this paper.

## MATERIALS AND METHODS

The experiments were performed on adult male Swiss mice bred randomly in our laboratory and given Purina fox chow and water *ad libitum*. The mice were 6 to 10 weeks old at the beginning of each experiment. The treated area was the back of the mice, which was shaved using electric hair clippers a day before the 1st treatment and then once a week throughout the experiments.

A potentially neoplastic state was induced in the epidermis by a single application of 7,12-dimethylbenz(a)anthracene (DMBA) in a 0.5% or a 1.5% concentration in mineral oil painted onto the back of a mouse with a No. 5 squirrel hair brush. The stage of the hair cycle at the time of treatment was not determined, as it was shown previously that the hair cycle does not affect the subsequent yield of tumors when the carcinogen is applied dissolved in mineral oil (3). Tumor en-

hancement, using the technics developed by Berenblum and Shubik (4, 5) and Mottram (10) was started 1 week later by repeated applications of croton oil in mineral oil at various frequencies of application, in various amounts, or for varying lengths of time. Croton oil was also painted onto backs using a No. 5 squirrel hair brush, except as noted in 1 experiment below.

DMBA was obtained from Eastman Organic Chemicals, Rochester, N.Y., croton oil from Boots Pure Drugs, Nottingham, United Kingdom or Bios Laboratories, New York, N.Y., and mineral oil from Plough Canada Ltd., Toronto, Ontario. None of these were further purified before use.

The mice were inspected once a week during the experimental period, and tumors were mapped on special charts as they appeared. A lesion was counted as a tumor if it was elevated and at least 1 mm in diameter. Lesions seen only once were counted, though very few tumors were noted fewer than 3 weeks in succession. Care was exercised not to remove visible tumors by the weekly shaving of the backs. The results are listed in the tables either as the total number of tumors that appeared on a group of mice over the duration of the experiment or, where needed, as new tumors appearing during particular weeks. A tumor index was also used in presenting the results; this was calculated for each experimental group by dividing the number of new tumors appearing in a week by the number of mice alive that week. These ratios were calculated for all weeks of the experiment and then added from the beginning of the experiment up to the time for which the tumor index is listed.

## RESULTS

Seventeen experimental groups were set up, as shown in Table 1, to test the effect of various durations of application of croton oil (twice a week for 7 weeks, for 14 weeks, or indefinitely); the effect of various frequencies of application of croton oil (3 times a week, or once every 3 weeks); or the effect of varying amounts of croton oil (as delivered by a large No. 5 brush, or by a small No. 00 brush) on the promotion of tumors initiated by a single application of a solution of DMBA. Appropriate control groups were included. Promotion in all these experiments was with a 5% solution of croton oil in mineral oil.

The first 6 groups demonstrated the effect of interrupted promotion. As shown previously (7, 8, 12, 13), the tumor yield fell when promotion was shortened (Groups 2 and 3). The interest in these groups was in the examination of the continuing effect of croton oil when treatments were stopped, as

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Table 1

Group No.	DMBA once	5% croton oil regime	No. of mice at start	Duration of promotion (weeks)	No. of mice at end	Total No. of tumors induced	Median week of appearance of tumors	Tumor index <sup>d</sup>
1	No	None	50	Life <sup>c</sup> (42.6)	0	0		0.0
2	0.5%	Twice a week for 7 weeks <sup>a</sup>	100	Life <sup>c</sup> (37.3)	0	209	8	2.0
3	0.5%	Twice a week for 14 weeks <sup>a</sup>	100	Life <sup>c</sup> (36.5)	0	669	11	7.4
4	0.5%	Twice a week until death <sup>a</sup>	50	Life <sup>c</sup> (37.2)	0	476	13	13.2
5	0.5%	None	50	Life <sup>c</sup> (41.2)	0	0		0.0
6	No	Twice a week until death <sup>a</sup>	50	Life <sup>c</sup> (35.8)	0	10	15	0.4
7	1.5%	Three times a week <sup>a</sup>	50	20	29	429	8	10.0
8	1.5%	Once in 3 weeks <sup>a</sup>	50	20	32	73	17	2.0
9	No	Three times a week <sup>a</sup>	50	20	22	3	16	0.1
10	No	Once in 3 weeks <sup>a</sup>	50	20	27	3	5	0.1
11	1.5%	None	50	20	26	0		0.0
12	0.5%	Three times a week <sup>b</sup>	50	40	16	146	15	3.9
13	0.5%	Once in 3 weeks <sup>b</sup>	50	40	28	70	32	1.9
14	No	Three times a week <sup>b</sup>	50	40	21	8	19	0.2
15	No	Once in 3 weeks <sup>b</sup>	50	40	17	0		0.0
16	1.5%	Twice a week #5 brush <sup>a</sup>	50	20	23	324	12	8.7
17	1.5%	Twice a week #00 brush <sup>a</sup>	50	20	26	275	14	7.7

Tumor yields in experiments where croton oil treatments were varied in duration, frequency, or amount. DMBA, 7,12-dimethyl(a)benzanthracene.

<sup>a</sup> Boots croton oil.

<sup>b</sup> Bios croton oil.

<sup>c</sup> Experiment continued until all animals died. Average life-span in weeks of mice in these groups from the beginning of the experiment is given in parentheses.

<sup>d</sup> For calculation see Materials and Methods.

summarized in Table 2. When contrasted with the effect of uninterrupted application of croton oil, it was seen that the marked effect of croton oil persisted for another 2 weeks, while some minor effect went on for a total of 5 weeks. Salaman (13) noted a similar effect. The other groups served as controls and behaved as expected.

Table 2

Week of experiment	Ratio of the number of new tumors appearing that week per number of mice at the beginning of the experiment					
	8	9	10	11	12	13
Promotion for 7 weeks <sup>a</sup>	0.90	0.32	0.09	0.04	0.03	0.01
Continuing promotion <sup>b</sup>	0.80	0.64	0.72	1.06	1.32	0.78
Week of experiment	15	16	17	18	19	20
Promotion for 14 weeks <sup>c</sup>	0.23	0.20	0.06	0.02	0.05	0.01
Continuing promotion <sup>b</sup>	0.42	0.40	0.10	0.12	0.08	0.22

Continuing appearance of new tumors following the last of a series of paintings with croton oil in contrast to the appearance of new tumors during continuing croton oil treatments. All mice were initiated by a single painting with 0.5% DMBA and were then promoted twice a week with 5% croton oil.

<sup>a</sup> Group 2 in Table 1.

<sup>b</sup> Group 4 in Table 1.

<sup>c</sup> Group 3 in Table 1.

The difference in the effect of giving croton oil 3 times a week or once in 3 weeks was demonstrated in Groups 7-15. Decreased frequencies of application decreased the yield of tumors, as was also noted by Merenmies (9) who used Tween 60, and by Boutwell (7) who used croton oil. The appearance of tumors was also markedly delayed, as was observed by Reissig and Graffi (11). In addition, it was possible to examine the persistence of the effect of croton oil as a tumor promoter in the groups given croton oil once every 3 weeks by merely looking at the number of new tumors appearing each week after the infrequent croton oil applications. Table 3 summar-

Table 3

	Number of new tumors after each application of croton oil			Total number of applications
	1 week later	2 weeks later	3 weeks later	
1st Experiment (Table 1, Group 8)	0	15	32	7
2nd Experiment (Table 1, Group 13)	17	36	16	14

Duration of the effect of each of a series of applications of croton oil given once every three weeks to initiated mice. Note (Table 1) that there are several other differences in these 2 experiments not relevant to the observations of this table.

izes this aspect of these experiments. It was apparent that any one of the applications of croton oil was effective in tumor enhancement for at least as long as 3 weeks. In one experiment (Group 8), the maximum of new tumors was in the 3rd week after each application; in the other (Group 13), in the 2nd week. In one experiment (Group 8), the 1st tumors appeared after the 2nd application of croton oil; in the other (Group 13), they appeared after the 5th application.

The effect of giving different amounts of croton oil at the same concentration and frequency was demonstrated in Groups 16 and 17 of Table 1. The amount delivered at each application differed by a factor of about 8. This was achieved by using brushes of 2 different sizes for the painting treatments, i.e., either the standard No. 5 brush used throughout these experiments or a smaller No. 00 brush. The amount of croton oil solution delivered by a brush was determined by painting a series of 10 preweighed pieces of paper with the brush and then reweighing them. The No. 5 brush was found to deliver 62.1 mg of croton oil solution (S.D., 12.5), and the No. 00 brush delivered 7.5 mg of the same solution (S.D., 3.9) per painting. There was no significant difference in tumor yield between the 2 groups treated with the 2 different brushes. Concentration would appear to be the more critical feature in the effect of a promoting agent than the amount given.

## DISCUSSION

The duration of the effect of croton oil in promoting the growth of tumors in animals pretreated with a carcinogen has been examined previously by Salaman (13). He noted that once tumors had begun to appear during 2-stage experiments, new tumors continued to appear for another 2-3 weeks after croton oil treatments were stopped. We have confirmed this observation. In addition, we demonstrated that, given the cumulative conditioning of the epidermis by croton oil, each single application in the series, as shown when croton oil was given once every 3 weeks, had a promoting effect lasting for at least 3 weeks. In the 2 experiments in which this was shown, the 1st tumors appeared, in one instance, after 2 applications of croton oil following initiation with 1.5% DMBA and, in the other instance, after 5 applications following initiation with 0.5% DMBA. As noted by others (7, 9, 11), fewer tumors appeared and the mean latent period was longer at lower frequencies of croton oil administration.

The results demonstrated that most new tumors appeared in about 2-3 weeks after an application of croton oil. This was, of course, only true once the absolute latent period of the appearance of tumors had passed. These observations are compatible with Berenblum's theory that clones of potentially neoplastic cells must grow to a colony of "critical size" in order to become truly neoplastic (2).

The concentration of croton oil applied to the skin was more important in tumor promotion than the amount. This could be because the larger amount used was in excess of maximal stimulation. Indeed, since there was only a moderate increase in tumor incidence by giving croton oil 3 times a week rather than twice a week (Groups 7 and 16, Table 1), this may be an explanation. On the other hand, if the area exposed is the

same, as it was in these experiments, concentration would be expected to be more important than the absolute amount.

In the experiments presented here, croton oil shortened the life-span of the animals (Groups 1-5, Table 1) and induced a small amount of ulceration of the skin. Such toxic effects may have been responsible for the observation of Klein (8) that fewer tumors were induced when croton oil was given 3 times a week than when given twice a week. We did not notice this effect, probably because of differences in the experiments. Klein used a different strain of mice (DBA), a different carcinogen (methylcholanthrene), and a different part of the skin (ears).

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