# The Carcinogenicity of Creosote Oil: Its Role in the Induction of Skin Tumors in Mice*

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Although exposure to creosote[1] was recognized 35 years ago as a carcinogenic hazard to man (5), it is only recently that its carcinogenic effect on mice has been noted and investigated. Cabot *et al.* (4) observed that tumors developed more rapidly in mice treated with a basic fraction of creosote oil and benzpyrene concurrently than in mice treated with benzpyrene alone. Woodhouse (13) found creosote a more potent carcinogen than a number of petroleum fractions tested. While the experiments reported here were carried out, Poel and Kammer (8) and Lijinsky, Saffiotti, and Shubik (7) demonstrated the carcinogenicity of creosote for mouse skin. In the present paper the carcinogenicity of creosote for mouse skin is confirmed, and its ability to initiate tumor formation when applied for a limited period prior to croton oil treatment is demonstrated.

## MATERIALS AND METHODS

Random-bred female, albino mice 8 weeks of age (±3 days) were used.[2] The animals were housed in screen-bottomed metal cages at 75±3° F. Water and a standard diet of Purina Laboratory Chow were given ad libitum. In addition to gross observations, body weight curves and survival records provided evidence that the condition of the mice was satisfactory.

Creosote[3] was used at full strength as obtained from the supplier. Croton oil[4] and DMBA[5] were dissolved in redistilled, thiophene-free benzene, and the concentration was expressed on a weight-volume basis. Solutions were protected from exposure to light and evaporation. Prior to the first application of test substances, the hair was shaved from the test area of the back of the mice with electric clippers. Because of the possibility of mechanical irritation and damage to papillomas, the mice were not shaved during the course of the experiment. The solutions were applied as a single 25-μl. drop to the mid-dorsal skin of each mouse at the time specified. The mice were inspected for tumors weekly. Only typical papillomas larger than 1 mm. in diameter were counted, care being taken to exclude hyperplasias and other miscellaneous lesions. The gross identifications of both benign and malignant tumors were confirmed periodically by microscopic examination.[6]

Seven groups of 30 female mice were formed from a common pool of animals born within 1 week. The mice of Group 1 were given no initial treatment, and after a delay of 1 week 1 drop (25 μl.) of undiluted creosote was applied twice weekly for the duration of the experiment. Group 2 received a single initial application of 75 μg. of DMBA per mouse and, beginning 1 week later, twice-weekly treatments with 25 μl. of benzene (solvent control). Group 3 received a single application of DMBA, as in Group 2, followed after 1 week with twice-weekly applications of 25 μl. undiluted creosote. Group 4 was treated with a single application of DMBA (as in Groups 2 and 3), followed after 1 week with 25 μl. of a 0.5 per cent solution of croton oil in benzene twice weekly. Group 5 was given no initial treatment, and after a delay of 1 week treatment with croton oil was begun (as in Group 4). Group 6 was treated with undiluted creosote twice weekly for 4 weeks only. There was no secondary treatment. Group 7 was treated with creosote twice a week for 4 weeks (as in Group 6), and after an interval of 1 week croton oil was applied as in Groups 4 and 5.

A summary of the schedule of treatments, together with length of the period of treatment and observation for each group, is shown in Table 1. The length of the period of observation of each group was determined by the carcinogenic response.

## RESULTS

Papillomas developed in Groups 1, 3, 4, 5, and 7 but not in Groups 2 and 6. The data, expressed in terms of the per cent of surviving mice bearing 1 or more papillomas, is plotted in Chart 1. The promoting action of creosote is illustrated in Chart 2 and the attempt to demonstrate initiating action in Chart 3. In the latter two charts the tumor incidence is expressed as the average number of papillomas per surviving

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[1] Creosote oil is a loosely defined industrial distillate of coal tar or of petroleum residues and therefore is of variable composition.

[2] These mice are of the Sutter strain and were obtained from the Holtzman Rat Company, Madison, Wis.

[3] Carbasota®, Barrett Chemical Co. The oil was described as fractions distilled from a high-temperature coke-oven tar in the boiling range of 200 to over 400° C. The product was specified to be crystal-free at 40° F.

[4] We are indebted to S. B. Pennick and Co. for a generous supply of croton oil.

[5] Distillation Products Industries, Rochester, N.Y.

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[6] We are indebted to Dr. H. P. Rusch for histological studies.

mouse. The development of carcinomas is shown in Chart 4.

*Papillomas.*—Creosote alone (Group 1, Charts 1 and 2) administered twice weekly caused rapid papilloma development. Between the 8th and 16th week the average number of papillomas per mouse increased from 0.3 to 5.0 and reached a maximum of 5.4 per mouse at the 28th week. A single application of 75 μg. of DMBA preceding the continuous creosote treatment (Group 3) shortened the time for the appearance of the first tumor by 4 weeks and resulted in a higher tumor incidence until the 24th week. Thus, the carcinogenic action of creosote was enhanced by pretreatment with DMBA, but the test did not provide satisfactory evidence for promoting activity of creosote oil in the absence of carcinogenesis.

TABLE 1

THE SCHEDULE OF TREATMENTS DESIGNED TO TEST FOR THE CARCINOGENICITY OF CREOSOTE AS WELL AS INITIATING AND PROMOTING ACTIVITY

| Group | Initial treatment | Secondary treatment* | Period of observation (weeks) |
|---|---|---|---|
| 1 | None | Creosote | 28 |
| 2 | 75 μg. DMBA once | Benzene | 56 |
| 3 | 75 μg. DMBA once | Creosote | 28 |
| 4 | 75 μg. DMBA once | Croton oil | 54 |
| 5 | None | Croton oil | 44 |
| 6 | Creosote, 4 wk. | None | 44 |
| 7 | Creosote, 4 wk. | Croton oil | 56 |

* The treatments were given in quantities of 25 μl. twice weekly for the period of observation. Creosote was applied full strength and croton oil as a 0.5 per cent (w/v) solution in benzene. There was an interval of 1 week between the initial and secondary treatments, and this delay was maintained in Groups 1 and 5.

Mice treated twice a week with croton oil only (Group 5, Charts 1 and 3) developed an average of only 0.1 papillomas per mouse by 28 weeks, but tumors continued to appear slowly throughout the experiment. At 44 weeks eight out of 26 surviving mice treated continuously with croton oil bore a total of thirteen papillomas, and one mouse bore an epithelial carcinoma that was confirmed by microscopic examination. In contrast, the mice treated with creosote twice a week for 4 weeks preceding croton oil treatment (Group 7) began to develop papillomas at the twelfth week, and by 28 weeks the average per mouse was 2.8. A maximum of 4.4 papillomas per mouse was attained by the 36th week. Papillomas developed most rapidly in the mice of Group 4 treated once with 75 μg. of DMBA followed by croton oil. The maximum value of six per mouse was reached by 16 weeks. Control Groups 2 (a single application of DMBA followed by benzene twice a week) and 5 (nine applications of creosote during the first 4 weeks) remained free of papillomas throughout the experiment. Although not so effective as 75 μg. of DMBA, as little as 0.25 μl. of creosote oil showed strong initiating action.

*Carcinomas.*—Mice of Group 1 (treated twice weekly with creosote) and Group 3 (creosote treatment preceded by DMBA) showed the same rate and time of appearance of carcinomas (Chart 4). In both cases carcinomas were first detected at 18 weeks, and the incidence reached 82 per cent of the effectual total by 28 weeks. A much slower rate of appearance of carcinomas resulted from a single application of DMBA followed by croton oil twice a week (Group 4). The first carcinoma was seen at 20 weeks, but it was not until the 42d week that an incidence of 80 per cent was attained. When the brief primary treatment with creosote was followed by croton oil (Group 7) no carcinomas were evident until the 34th week. Thereafter the incidence rose rapidly so that by the 44th week 46 per cent of the mice had carcinomas. No malignant skin tumors were seen before the end of the experiment (44 weeks) in Group 2 or 6.

TABLE 2

THE AVERAGE INDUCTION TIME FOR PAPILLOMAS AND CARCINOMAS

| Group | Treatment | Induction time: Papillomas (wk.) | Induction time: Carcinomas (wk.) | Ratio of induction times* |
|---|---|---|---|---|
| 1 | Creosote alone | 20 | 26 | 1.3 |
| 2 | DMBA once | | | |
| 3 | DMBA+creosote | 16 | 23 | 1.4 |
| 4 | DMBA+croton oil | 10 | 36 | 3.6 |
| 5 | Croton oil alone | | | |
| 6 | Creosote 4 wk. only | | | |
| 7 | Creosote+croton oil | 20 | 44 | 2.2 |

* The length of the induction time for carcinoma formation divided for papilloma formation. The average induction time is defined as the time required for the production of tumors in 50 per cent of the animals.

*Tumor induction time.*—The relationship between the rate of appearance of papillomas and carcinomas may be seen to advantage by considering the average induction times shown in Table 2. The average induction time is defined as the time required for the appearance of tumors in 50 per cent of the mice and is generally known as latent period (1). A single application of DMBA followed by croton oil was the most effective procedure to elicit papillomas (50 per cent of the mice bore one or more papillomas at 10 weeks [Chart 1]), but carcinomas appeared slowly, reach-

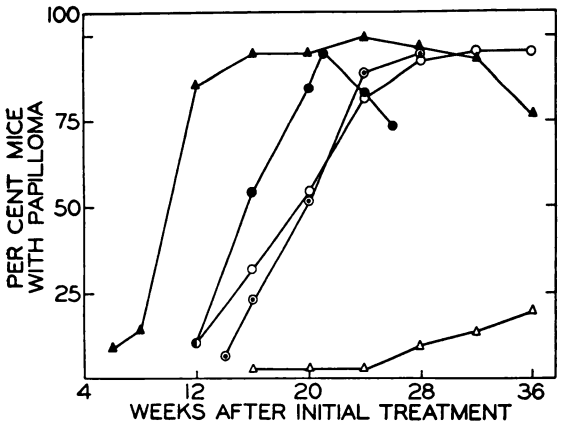


CHART 1.—The development of papillomas in mice treated with DMBA, creosote, or croton oil, alone or in combination. The percentage of surviving mice bearing one or more papillomas is plotted against time (in weeks) from the start of the experiment (e.g., the time of the single application of DMBA).

The groups and test substances are indicated as follows:

⊙——⊙ group 1: creosote alone.
●——● group 3: DMBA once followed by creosote.
▲——▲ group 4: DMBA once followed by croton oil.
△——△ group 5: croton oil alone.
○——○ group 7: creosote for 1 month followed by croton oil.

No tumors developed in the mice treated with DMBA once (Group 2) or with creosote alone for 1 month (Group 6). In the other groups, creosote or croton oil was applied twice weekly for the duration of the experiment.

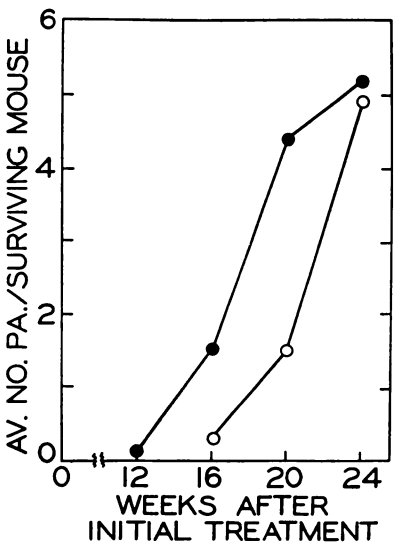


CHART 2.—A test for promoting activity of creosote oil.

The average number of papillomas per surviving mouse is plotted against the elapsed time from the start of the experiment.

The groups and test substances are indicated as follows:

○——○ group 1: creosote alone.
●——● group 3: DMBA once followed by creosote.

No tumors developed in the mice treated with DMBA once alone (Group 2).

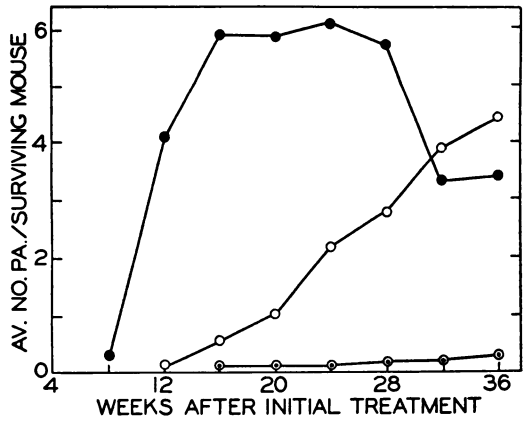


CHART 3.—The initiating action of creosote oil. The group in which tumors were initiated by a single application of 75 μg. of DMBA per mouse is included for comparison. The average number of papillomas per surviving mouse is plotted against the elapsed time from the start of the experiment.

The groups and test substances are indicated as follows:

●——● group 4: DMBA once, followed by croton oil.
⊙——⊙ group 5: croton oil alone.
○——○ group 7: creosote for 1 month, followed by croton oil.

No tumors developed in the mice treated with DMBA once (Group 2) nor with creosote alone for 1 month (Group 6).

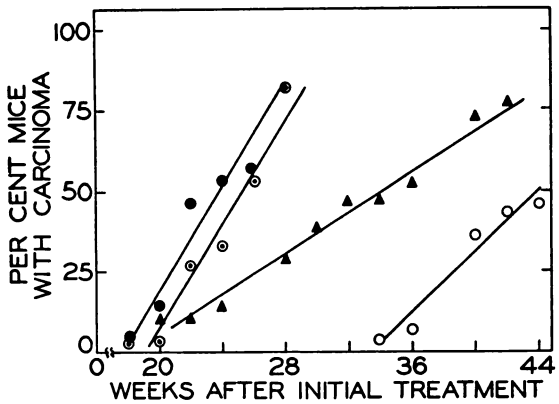


CHART 4.—The development of carcinomas in mice treated with DMBA, creosote, or croton oil, alone or in combination. The per cent of mice bearing carcinoma* is plotted against the elapsed time from the start of the experiment.

The groups and test substances are indicated as follows:

⊙——⊙ group 1: creosote continuously alone.
●——● group 3: DMBA once, followed by creosote.
▲——▲ group 4: DMBA once, followed by croton oil.
○——○ group 7: creosote for 1 month, followed by croton oil.

No carcinomas developed in the mice treated with DMBA once (Group 2) with croton oil alone (Group 5), nor with creosote alone for 1 month (Group 6).

* The percentage of mice with carcinoma is expressed as the number of mice with carcinoma (including those already dead with carcinoma) multiplied by 100 and divided by the number of mice alive in the respective group at the time the first carcinoma appeared in the experiment.

ing 50 per cent at 36 weeks. In contrast, in the two groups in which the long continued application of creosote was the predominant feature, the papilloma incidence did not reach 50 per cent until 16–20 weeks, while the induction time for carcinomas was only 23–26 weeks. The induction time for carcinomas was the longest (44 weeks) in mice treated with creosote for 4 weeks followed by croton oil, while that for papilloma formation was 20 weeks. It is apparent that there is no fixed relationship between the lengths of the induction times for papilloma and carcinoma formation.

## DISCUSSION

Poel and Kammer (8) and Lijinsky, Saffiotti, and Shubik (7) have demonstrated the carcinogenicity of creosote oil for mouse skin. The present experiments confirm this observation. Since creosote is carcinogenic for mouse skin it must by definition have both initiating and promoting properties (2, 6). The former has now been satisfactorily demonstrated (Chart 3). An attempt was made to demonstrate the tumor-promoting activity of creosote by applying creosote twice weekly after a single application of DMBA. However, treatment with creosote alone without prior DMBA treatment produced almost maximal tumor response. Lijinsky *et al.* (7) also failed to demonstrate a tumor-promoting action by undiluted creosote. On the other hand, they found that treatment with a 10 per cent solution of creosote in acetone following a single application of DMBA gave rise to tumors, but they did not test 10 per cent creosote alone for carcinogenicity. It would be of interest to determine whether a tumor-promoting effect could be demonstrated by testing diluted creosote with and without pretreatment with DMBA.

The ability of croton oil to induce papillomas in untreated mice was again confirmed (3, 9, 10).

Rous (11) first called attention to the neoplastic response in mice resulting from the use of wooden cages "impregnated with a commercial wood preservative" identified verbally as creosote. Shubik (12) reported the appearance of papillomas in untreated mice from dealer's stock, but the cause of these tumors remained obscure. A similar observation was recorded by Boutwell *et al.* (3). It is now apparent that these "spontaneous" papillomas were caused by housing the mice in creosoted wooden boxes.[7] Furthermore, the present experiment explains the unusual sensitivity of such mice to the promoting effect of croton oil (3). For example, as little as 0.25 ml. of creosote per mouse, divided into nine applications over a period of 1 month, resulted in a high yield of papillomas and carcinomas after subsequent applications of croton oil. No doubt smaller quantities of creosote could be shown to be effective. It is obvious that mice exposed to creosote are unsuitable for use in experimental oncology.

The incidence of papillomas was presented in terms of the percentage of mice bearing one or more papillomas as well as the average number of papillomas per surviving mouse. A comparison of the two methods of expression shows the latter to be a better measure of response in certain cases, especially in the groups treated with DMBA followed by croton oil and creosote followed by croton oil. Even after 90 per cent of the mice in these groups bore papillomas, the total number of papillomas continued to increase with time.

The capacity of DMBA followed by croton oil to elicit papillomas rapidly and in large numbers was outstanding among the combinations reported. However, this same combination was relatively weak in causing carcinomas. In contrast, in the groups treated with creosote continuously (with or without initial DMBA), papillomas began to appear later, accumulated more slowly, and continued to accumulate after the 16th week, while carcinomas began to appear in the 18th week and accumulated more rapidly than in the DMBA plus croton oil group. The differences in the efficacy of the experimental treatments to elicit papillomas and carcinomas was best illustrated by the great differences in the ratio of the tumor induction time of the two lesions as defined in Table 1. It is apparent that different factors must be involved in the formation of the two lesions, and that further work is required to understand the mechanisms involved. Both the initiating agent and the promoting agent are important in determining the length of the tumor induction time as well as the type of tumor produced.

## SUMMARY

1. Mice were treated with 9,10-dimethyl-1,2-benzanthracene (DMBA), creosote oil, or croton oil, alone or in several combinations of two of the agents. The incidence of papillomas and carcinomas as well as the tumor induction time of each were reported.

2. The effective nature of creosote as a skin carcinogen was confirmed, and its ability to act

[7] The dealer, Mr. Arthur Sutter, Springfield, Mo., described (personal communication) the operation of his colony in detail, including the methods used to treat the wooden cages with Carbasota brand of creosote. After he was informed of the effect on the mice he stated that he ceased using creosote in December, 1956.

as an initiating agent prior to applications of croton oil was shown.

3. The average induction times for papillomas and carcinomas by the different treatments were found to vary independently.

## REFERENCES


1. BERENBLUM, I. A System of Grading Carcinogenic Potency. Cancer Research, 5:561–64, 1945.
2. BERENBLUM, I., and SHUBIK, P. A New, Quantitative Approach to the Study of Stages of Chemical Carcinogenesis in the Mouse's Skin. Brit. J. Cancer, 1:383–91, 1947.
3. BOUTWELL, R. K.; BOSCH, D.; and RUSCH, H. P. On the Role of Croton Oil in Tumor Formation. Cancer Research, 17:71–75, 1957.
4. CABOT, S.; SHEAR, N.; and SHEAR, M. J. Studies in Carcinogenesis; Development of Skin Tumors in Mice Painted with 3,4-benzpyrene and Creosote Oil Fractions. Am. J. Path., 16:301–12, 1940.
5. COOKSON, H. A. Epithelioma of Skin after Prolonged Exposure to Creosote. Brit. M. J., 1:368, 1924.
6. FRIEDEWALD, W. F., and ROUS, P. The Initiating and Promoting Elements in Tumor Production. J. Exper. Med., 80:101–26, 1944.
7. LIJINSKY, W.; SAFFIOTTI, V.; and SHUBIK, P. A Study of the Chemical Constitution and Carcinogenic Action of Creosote Oil. J. Nat. Cancer Inst., 18:687–92, 1957.
8. POEL, W. E., and KAMMER, A. G. Experimental Carcinogenicity of Coal Tar Fractions: The Carcinogenicity of Creosote Oils. J. Nat. Cancer Inst., 18:41–50, 1957.
9. RITCHIE, A. C. Epidermal Carcinogenesis in the Mouse by Intraperitoneally Administered Urethane Followed by Repeated Applications of Croton Oil. Brit. J. Cancer, 11:206–11, 1957.
10. ROE, F. J. C. The Development of Malignant Tumors of Mouse Skin after "Initiating" and "Promoting" Stimuli. III. The Carcinogenic Action of Croton Oil. Brit. J. Cancer, 10:72–78, 1956.
11. ROUS, P. Influence of Hereditary Malformations on Carcinogenesis in "CREW" Mice and Deer Mice of Hairless Strains. Proc. Am. Assoc. Cancer Research, 2:143, 1956.
12. SHUBIK, P.; SPENCER, K.; and DELLA PORTA, G. The Occurrence of Skin Tumors in Untreated Mice from Dealer's Stock. J. Nat. Cancer Inst., 19:33–36, 1957.
13. WOODHOUSE, D. L., and IRWIN, G. O. The Carcinogenic Activity of Some Petroleum Fractions and Extracts. J. Hyg., 48:121–34, 1950.

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