

# The Carcinogenicity of Creosote Oil: The Induction of Lung Tumors in Mice\*

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Mice, obtained when 8–12 weeks old from a commercial breeder<sup>1</sup> and kept without treatment until they were 6 or more months old, were found to have a high incidence of lung adenomas. In contrast, the progeny of the same mice, born and raised in this laboratory, had a low incidence of lung tumors after a similar period. It was already known that the breeder from whom the mice were obtained housed the mice in wooden boxes treated with creosote oil<sup>2</sup> and that this led to the development of skin tumors in untreated mice (18) and also increased the susceptibility of mice to the induction of skin tumors (5). Therefore, creosote was tested and found both to increase the percentage of mice bearing lung tumors and the number of adenomas borne by individual mice over the control level.

In the first experiment creosote was applied to the skin of weanling mice repeatedly in quantities sufficient to cause both skin and lung tumors to appear. In the second experiment the quantity of creosote applied to the skin, though insufficient to cause skin tumors, was nevertheless effective in giving rise to lung tumors.

## MATERIALS AND METHODS

The general methods were the same as those described in the preceding paper (5). The creosote oil, known as Carbasota (®), was obtained from the Barrett Chemical Co. It was described as material

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<sup>1</sup> Arthur Sutter, Springfield, Mo.

<sup>2</sup> Mr. Sutter described (personal communication) the operation of his colony in detail, including the methods used for the treatment of the wooden boxes with Carbasota brand of creosote. After he was informed of the effect on the mice, he ceased using creosote in December, 1956.

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in the boiling range of 200° to over 400° C., distilled from a high-temperature coke-oven tar. The product was crystal-free at 40° F.

To determine the incidence of lung tumors, the lungs were removed from freshly killed mice and the lobes separated. Only the tumors visible on the surface of the lobes were counted, and the naked-eye diagnosis of these was in many cases checked by histological examination.

## RESULTS

*Preliminary observation.*—A total of 788 adenomas were counted on the lungs of 138 mice (an average of 5.8 adenomas per mouse) at 6–8 months of age; these mice had been obtained from the commercial breeder when 2–3 months old. They were received in four separate consignments over a period of 10 months.

Some of the same mice were allowed to breed in metal cages in this laboratory. The resulting progeny at the same age had an average of less than 0.5 adenomas per mouse (twelve adenomas in 26 mice).

*Experiment 1.*—In this experiment, which was to test whether creosote causes lung tumors, mice of the fourth generation raised in the laboratory from the dealer's mice were used. One batch of these fourth-generation mice was put to breed in stainless steel boxes on pine shavings. Of the resulting progeny, 24 were kept for 8 months as untreated controls (Group 1). The remaining 25 were treated twice weekly with 1 drop (25  $\mu$ l.) of creosote oil applied to the skin of the back from the age of 3 weeks until 6 months and then kept for 2 months without treatment (Group 2). A second batch of fourth-generation mice was put to breed in wooden cages which had been thoroughly impregnated with creosote oil. Group 3 was made up of 29 young born in the creosoted cages and kept in these cages for the duration of the experiment. In addition, creosote was applied to the skin of these mice (1 drop per mouse twice a week) for 5 months after weaning. The mice were then kept for 3 months without treatment.

The incidence of lung adenomas at 8 months is

shown in Table 1. Mice of Group 1 had an average of less than 0.5 adenomas per mouse, those of Group 2 had almost six per mouse, and those of Group 3 almost eleven per mouse.

Groups 2 and 3 combined totaled 53 mice; of these five bore skin tumors but no lung tumors, nine had lung tumors but no skin tumors, and 39 bore both skin and lung tumors.

*Experiment 2.*—Because in the first experiment the mice were exposed to large amounts of creosote, the effect of a much smaller dose was tested. Random-bred albino mice were obtained from the Holtzman Rat Co., Madison, Wis.<sup>3</sup> This breeder uses only metal cages for breeding, and the breeding environment is similar to that maintained in this laboratory. The mice were divided into groups of 30 and housed in wire-bottomed metal cages. The experimental treatments were begun when the mice were 8 weeks old. One group (Group 2) was

tration of lung carcinogens have proved effective including subcutaneous (1), intraperitoneal (12), oral (6), intravenous (4), direct implantation into lung (2), and percutaneous (11). In fact, of all tissues, the lungs of susceptible mice may be the most responsive tissue to a variety of carcinogens. This is illustrated by the mice of Experiment 2, Group 2, in which 37 lung tumors but no skin tumors were found in response to nine applications of creosote oil to the same area of skin. Furthermore, Andervont (1) reported that more mice of strain A, given a subcutaneous injection of 1,2,5,6-dibenzanthracene, developed lung tumors than subcutaneous tumors and that the lung tumors appeared earlier.

In view of the finding that tar applied to the skin gave rise to lung tumors (11), it was not surprising that creosote oil was found to have a similar action. However, it is of interest that none of the constituents isolated from a sample of creosote

TABLE 1  
THE EFFECT OF CREOSOTE TREATMENT OF THE SKIN  
ON THE INCIDENCE OF LUNG ADENOMAS

Experi- ment	Group	Treatment	No. mice	No. adenomas	Av. no. adenomas per mouse
1	1	None (control group)	19	9	0.5
	2	Creosote	24	139	5.8
	3	Creosote and creosot- ed cages	29	315	10.8
2	1	Croton oil or benzene (control group)	50	15	0.3
	2	Creosote	23	37	1.6

treated twice weekly for 4 weeks (9 times in all) with 1 drop of creosote to the skin of the back of each mouse and thereafter was kept under observation without treatment. No untreated controls were available in this experiment, which was designed for the induction of skin tumors (5). However, a control was provided by mice that had been treated for the duration of the experiment with either 1 drop of 0.5 per cent croton oil twice weekly or with 1 drop of purified benzene twice a week. There were in all 50 mice so treated (Group 1).

At 10 months only fifteen adenomas were seen in the 50 survivors of Group 1. On the other hand, the 23 survivors of Group 2 bore a total of 37 lung tumors (see Table 1, Experiment 2). No skin tumors developed in any of these mice.

## DISCUSSION

Primary lung tumors have been induced in mice by a variety of agents. These include tar (11), hydrocarbons (1), nitrogen and sulfur mustards (7, 8), irradiation (10), aminoazotoluene (3), and urethan (12). A number of routes for the adminis-

oil by Lijinsky and his colleagues (9) (carbazole, benzanthracene, chrysene, fluoranthene, pyrene, anthracene, phenanthrene), has, as far as we know, been shown to give rise to lung tumors either when applied separately to the skin or when given by any other route. It is, of course, possible that one or more of these substances is carcinogenic for mouse lung but that the effect has been missed, or that the constitution of the creosote used by us was very different from that of Lijinsky *et al.* Alternatively, the carcinogenic effect of creosote on lung may be the result of the combined action of two or more of its constituents, or it may be due to unidentified constituents. It is unlikely that the oil contained sufficient benzpyrene to account for its activity (9, 13).

A previous report (5) indicated that mice bred in creosoted wooden cages were more responsive to skin tumor-inducing treatments than mice bred in metal cages. The present report indicates that exposure to creosote increases the incidence of lung tumors. It also suggests that exposure to small amounts of creosote oil during the first few weeks of life may have a disproportionately large effect

<sup>3</sup> The colony was established from Sutter stock.

on the subsequent lung tumor incidence. It is well known that lungs of embryonic and very young mice are more susceptible to urethan-induced carcinogenesis (15).

There is, of course, no reason to think that the oncogenic effect of creosote on the lungs of mice is secondary to that on skin, or *vice versa*. The histological appearances of the two tumors are quite different. Moreover, each type of tumor may be induced separately: urethan applied to the skin induces lung tumors but no skin tumors (17);  $\beta$ -propiolactone produces skin tumors but no lung tumors (14). On the other hand, it is probable that tissues other than skin and lung are affected by exposure to creosote, and it is possible that the tumor incidence of other tissues is increased.

Creosote oil has been utilized as a wood preservative and disinfectant in many animal colonies, and it has been only within the past few years that its use has been abandoned by some of the larger commercial breeders of mice in the United States<sup>4</sup> (16). Every effort should be made to discourage the use of creosote oil in all other animal colonies.

#### SUMMARY

1. Mice obtained from a commercial supplier were found to have a very high incidence of lung tumors, whereas the progeny of the same mice bred in this laboratory in stainless steel cages had a low incidence. The use by the animal supplier of creosoted wooden cages for breeding was the suspected cause for the high tumor incidence in the parent mice.

2. Creosote applied to the skin of mice led to a high incidence of both skin and lung tumors. Mice reared in creosoted wooden boxes and also painted with creosote had a higher lung tumor incidence than mice reared in metal cages and painted with creosote.

3. In a second experiment it was shown that quantities of creosote too small to cause skin tumors were nevertheless effective in giving rise to lung adenomas.

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<sup>4</sup> Personal communications.

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