Mouse Mammary Carcinogenesis by Ethyl and Butyl Carbamates[1] (34228)

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(Introduced by P. Shubik)

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The interaction of effects of chemical carcinogens and intrinsic factors determining the frequency with which a given tumor appears in an animal population is of great interest since this situation is also found in human populations. In the present paper the carcinogenic effect for mouse mammary glands by ethyl carbamate (urethan) and butyl carbamate administered separately and in combination is described and the effects of varying fractionation of the dose of the first are also studied.

The incidence of mammary carcinomas in mice is related to the genetic constitution of the animals, level of estrogens in the circulating blood, and the presence of a viral particle, the Bittner factor (1–3). The administration of methylcholanthrene to mice increases the frequency and shortens the latent period of development of mammary tumors (4–8). Using the LAF 1 strain of mice, the frequency of mammary carcinomas is increased by methylcholanthrene and ethyl carbamate when an overproduction of ovarian hormones is provoked by implantation of hypophysis in the kidney (9).

Ethyl carbamate is a potent carcinogen for mice, rats, and hamsters (10, 11). Butyl carbamate does not increase the number of pulmonary tumors in susceptible strains of mice (12). When ethyl carbamate and butyl carbamate are simultaneously administered in equimolecular doses to mice the "initiating" effect on skin carcinogenesis of the former is inhibited (13).

Material and Methods. Virgin female mice of the strain C_3H, originally obtained from Jackson Memorial Laboratories in 1946, and since bred by sister–brother mating in our laboratory, were used. All animals were 8–10 weeks of age, and of 18 g average weight. They were kept in plastic cages with wood shavings and were fed Cia. Molinera San Cristobal pellets for mice and water *ad libitum*.

Ethyl carbamate and butyl carbamate (Fisher Scientific Co.) as 1% solutions in distilled water were injected intraperitoneally. Croton oil (Laboratorio de Farmacologia, Escuela de Medicina Veterinaria, Universidad de Chile) as an 0.5% solution in acetone (Fisher Scientific Co.) was used for administering as one drop of the solution on the skin of the interscapular area.

Experiments. In a first experiment 90 animals were divided into 3 experimental groups: Group one was inoculated with a total dose of 30 mg of ethyl carbamate fractionated into 10 daily doses of 3 mg each. Group two was inoculated with the same total dose of ethyl carbamate fractionated into 3 daily doses of 10 mg each. Group three served as a control and was kept untreated. All groups were painted on the skin with one drop of croton oil twice a week for 20 weeks.

In a second experiment 120 mice were divided into four experimental groups of 30 animals each. Group one was inoculated with ethyl carbamate in a total dose of 30 mg fractionated into 6 daily doses of 5 mg each. Group two was inoculated with equimolecular quantities of ethyl carbamate and butyl carbamate (30 mg of ethyl carbamate and 39.57 mg of butyl carbamate) fractionated into six daily doses of 5 mg of ethyl carbamate and 6.595 mg of butyl carbamate each. Group

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TABLE I. Mammary Tumors in Mice Receiving Ethyl Carbamate.[a]

Group	Daily dose	No. of animals	26 Weeks TBA	%	TT	52 Weeks TBA	%	TT	Animals (%) with lung metastasis
Control		19	2	10.5	2	7	36.8	9	14.2
Ethyl carbamate	10 mg × 3	28	11	39.2	14	19	67.8	26	42.1
Ethyl carbamate	3 mg × 10	28	6	21.4	8	18	64.2	27	38.8

[a] TBA = tumor bearing animals; TT = total of tumors. All animals were painted with croton oil on the skin.

three was inoculated with a total dose of 39.57 mg. of butyl carbamate fractioned into six daily doses. Group four was kept untreated as a control.

A few animals in each group died soon after the total dose of ethyl carbamate or butyl carbamate had been given. These are not included in the final results. In surviving animals the appearance of mammary tumors was recorded as soon as noted, and those with tumors that were moribund were sacrificed. Animals that did not develop tumors were sacrificed after 1 year's observation.

All animals were autopsied and lung tumors were counted under the stereoscopic microscope. Tumors and lungs were studied histologically.

Results. Ethyl carbamate. The administration of ethyl carbamate increases the frequency of mammary tumors (Fig. 2) from 36.8% in the control group to 67.8% in the treated group in the first experiment (Table I) t: 4.1 $p<0.001$. In the second experiment the carcinogenic effect of ethyl carbamate is also evident (36% in the control group compared with 50% in the experimental group) (Table II) t: 1.8 $p < 0.05$. When a massive dose of ethyl carbamate is administered instead of a fractionated dose, the latent period of appearance of tumors is shortened, and accelerated development of tumors is observed, as can be seen from the results at 26 weeks (Table I). However, at 52 weeks this difference between fractionated and massive dose is inapparent. The same phenomenon can be observed in Fig. 1.

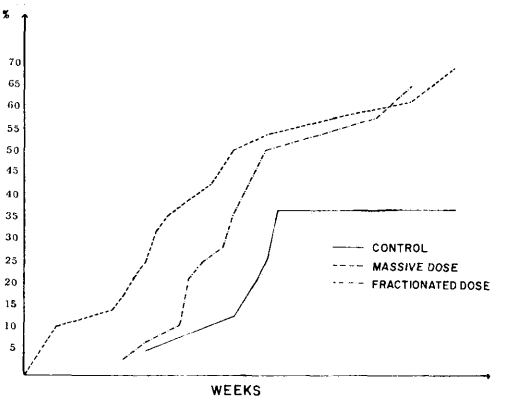

FIG. 1. Mammary carcinomas in mice receiving 30 mg of ethyl carbamate.

Butyl carbamate. Butyl carbamate significantly increases the percentage of animals that develop mammary tumors: 59.2% compared with 36.3% in the control group, (Ta-

TABLE II. Mammary Tumors in Mice Receiving Ethyl and Butyl Carbamate.[a]

Group	Daily dose	No. of animals	26 Weeks TBA	%	TT	52 Weeks TBA	%	TT	Animals (%) with lung metastasis
Control		22	1	4.5	1	8	36.3	8	12.5
Ethyl carbamate	5 mg × 6	24	4	16.6	4	12	50	16	58.3
Butyl carbamate	6.595 mg × 6	27	3	11.1	3	16	59.2	17	24.9
Butyl carbamate + ethyl carbamate	6.595 mg × 6 5 mg × 6	24	5	20.8	6	17	70.8	18	23.5

[a] TBA = tumor bearing animals; TT = total of tumors.

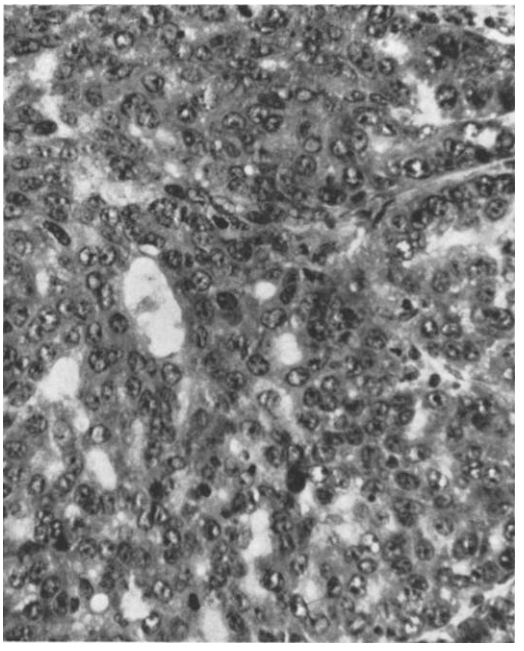

FIG. 2. Mammary carcinoma.

ble II) t: 3.1 $p < 0.01$. Butyl and ethyl carbamate administered simultaneously seem to have an additive effect, 70% of the animals developing tumors, a higher incidence than in groups given ethyl carbamate or butyl carbamate alone.

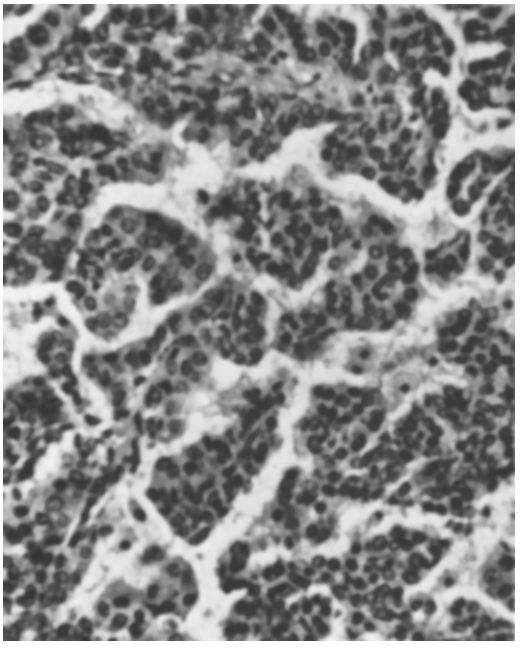

FIG. 3. Metastasis of a mammary carcinoma.

Croton oil. Animals skin painted with croton oil in acetone solution, after administration of ethyl carbamate, did not develop skin tumors. The percentage of animals with mammary tumors in this group was 67% (Table I) compared with 50% in those in which painting with croton oil was not done. This result is not significant: t: 1.28 $p > 0.05$.

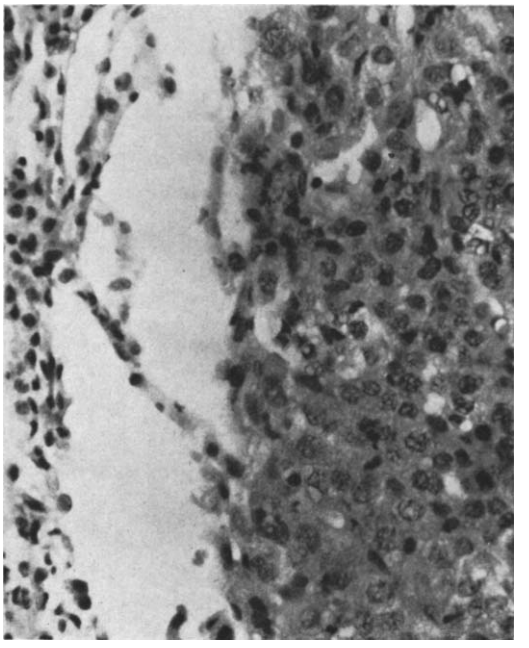

FIG. 4. Lung adenoma.

Lung tumors. The frequency of lung adenomas in the present experiments was extremely low (Fig. 4). They appeared in only one group treated with a massive dose of ethyl carbamate; these lungs were discarded when studying the frequency of lung metastasis. Lung metastasis in animals bearing carcinomas of breast was 12.5% in control groups of the first experiment (Table I) and 14.2% in the second (Table II) (Fig. 3). Ethyl carbamate in massive and fractionated doses and butyl carbamate significantly increased the percentages of metastasis in animals with breast tumors.

Discussion. It was demonstrated that ethyl carbamate and butyl carbamate are effective

carcinogens in increasing the yield of mammary tumors in intact female mice of the C_3H strain. These results are comparable with those obtained by others with ethyl carbamate and also with methylcholanthrene (4–6). Administration of a massive dose of ethyl carbamate compared with a fractionated dose appears to shorten the latent period of tumor development and increase the speed with which the tumors develop, although the total number of tumors obtained remains the same. The simultaneous administration of butyl carbamate and ethyl carbamate increases the frequency of tumors to a total higher than that obtained when the drugs are administered separately.

These results differ from previously observed inhibition of carcinogenic effect when two carcinogens are administered simultaneously (14) or when a carcinogen is given with a compound of similar molecular structure (15, 16).

The effect of both drugs on the frequency of metastasis is evident. By careful histological studies the possibility of confusing lung adenomas with metastasis of breast carcinomas was eliminated. The histologic pattern and the vascular localization in many cases was important in distinguishing lesions. We have no satisfactory explanation for the phenomenon.

Summary. Experiments examining the effect of ethyl carbamate and butyl carbamate on mammary carcinogenesis are reported. Virgin female C_3H mice receiving ethyl carbamate or butyl carbamate showed a higher incidence of tumors than the controls. The action of ethyl carbamate was the same when given in massive or fractionated dose but a massive dose shortened the latent period of tumor development. Simultaneous administration of ethyl carbamate and butyl carbamate had a summational effect. Administration of croton oil to animals receiving ethyl carbamate did not provoke the appearance of skin tumors. A higher number of metastases was observed in animals bearing breast tumors when ethyl carbamate or butyl carbamate was administered.

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