

# The Response of Mice to the Simultaneous Application of Two Different Carcinogenic Agents

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In a previous paper we reported on results of experiments in which rats were treated with two differently acting carcinogens (2). That study was made in order to investigate the possible influence of one such agent on the action of another. No interaction between the carcinogens was found. The present report deals with similar experiments on albino mice.

## MATERIALS AND METHODS

Animals used in these experiments were albino mice of our own breed, 6 to 8 weeks old. The spontaneous tumor rate in this stock is 0.5 per cent at the age of 1 year if lung tumors are not included. The latter have been observed in 5 per cent of the animals autopsied at the age of 4 months, in 15 per cent of mice 6 months old and in 25 per cent of mice at 1 year of age.

The methylcholanthrene was dissolved in 0.1 ml. of olive oil and injected subcutaneously. A single injection was given to each animal. Urethane and *p*-dimethylaminoazobenzene were mixed with the diets in the amounts indicated in Table I and in the same manner as previously described (2). The carcinogenic diet was given from the day of injection of methylcholanthrene through the whole experimental period. At the end of 4 months the animals were killed and autopsied and examined for local tumors. The lungs were fixed in Bouin solution and formol as previously described (3) and examined for the presence of adenomas.

## RESULTS

In Table I, a summary of the animals with local tumors and with pulmonary adenomas is given. In no case was a significant difference observed between the tumor incidence in the groups treated with 2 carcinogens as compared with the control groups treated with only 1 of the carcinogens.

The treatment with *p*-dimethylaminoazobenzene never caused detectable liver damage under the experimental conditions. Nevertheless, the dye, when fed with the diet, can be considered to

TABLE I: LOCAL AND LUNG TUMORS OBSERVED IN MICE 4 MONTHS AFTER BEGINNING OF TREATMENT

Treatment	Effective total	Local tumors %	Lung tumors, %
0.9 mgm. MC* subcu. + diet cont'g. 0.75% ethyl urethane	28	87	89
0.45 mgm. MC subcu. + diet cont'g. 0.15% ethyl urethane	75	76	95
0.225 mgm. MC subcu. + diet cont'g. 0.15% ethyl urethane	27	46	96
0.45 mgm. MC subcu. + diet cont'g. 0.015% <i>p</i> -dimethylaminoazobenzene	31	74	23
0.225 mgm. MC subcu. + diet cont'g. 0.015% <i>p</i> -dimethylaminoazobenzene	23	39	43
CONTROLS			
0.9 mgm. MC subcu.	164	80	45
0.45 mgm. MC subcu.	78	67	32
0.225 mgm. subcu.	68	41	31
Diet cont'g. 0.75% ethyl urethane	50	—	98
Diet cont'g. 0.15% ethyl urethane	14	—	100
Diet cont'g. 0.015% <i>p</i> -dimethylaminoazobenzene	22	—	25

\* MC = methylcholanthrene

be carcinogenic for the strain of mice used, for the data in Table II show that a significant rise in the rate of pulmonary adenomas over the spontaneous rate can be observed after 75 days of feeding the carcinogenic dye. Andervont, White and Edwards (1) have shown that *o*-aminoazotoluene produces lung tumors when fed to mice, but they did not observe such an effect with *p*-dimethylaminoazobenzene in their experiments with strain C mice.

TABLE II: LUNG TUMORS OBSERVED IN MICE 75 DAYS AFTER BEGINNING OF FEEDING ETHYL URETHANE OR *p*-DIMETHYLAMINOAZOBENZENE

Treatment	Effective total	Lung tumors, %
Diet cont'g. 0.015% <i>p</i> -dimethylaminoazobenzene	50	22
Diet cont'g. 0.15% ethyl urethane	75	59
Controls	57	5

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## DISCUSSION

The results of experiments with mice confirm those obtained previously with rats (2), namely that *p*-dimethylaminoazobenzene or ethyl urethane do not influence the number of tumors elicited by methylcholanthrene. The number of mice developing local tumors after a subcutaneous injection of methylcholanthrene was the same whether the animals received simultaneously another carcinogen with the diet or whether they were kept on the stock diet without further treatment.

Under the experimental conditions it was not possible to decide whether the action of injected methylcholanthrene in producing pulmonary adenomas in mice is additive to the action of urethane as the number of animals developing this kind of tumor was close to 100 per cent. In 2 series treated with methylcholanthrene and fed a diet containing *p*-dimethylaminoazobenzene no such additive action could be detected but the number of mice was not sufficient to draw definite conclusions.

The results indicate that in general 2 different carcinogens act independently. This observation does not necessarily exclude the possibility that under special conditions an interaction may be observed. It is known that carcinogenic hydrocarbons may have a retarding action on growth of transplanted tumors (7) and recently Murphy and Sturm (6) found that ethyl urethane inhibits the development of lymphatic tumors and leukemia in rats. This compound has a definite carcinogenic action in rats (4).

Indeed, we found recently that mice that had received *p*-dimethylaminoazobenzene with the diet for 6 weeks previous to a single injection of urethane developed fewer pulmonary adenomas than did the controls (5). Therefore care should be taken not to accept the independent action of simultaneously applied differently acting carcinogens as a rule without having studied each special case.

The present experiments show that feeding *p*-dimethylaminoazobenzene at a level of only 0.015 per cent for 75 days raises the spontaneous

rate of pulmonary adenomas to a significant degree. No such action of the azo dye has been reported previously as far as we know, although the related compound, *o*-aminoazobenzene, has been shown to stimulate the development of pulmonary tumors in mice (1).

## SUMMARY

Albino mice were injected subcutaneously with methylcholanthrene and were given a diet containing sufficient ethyl urethane to produce pulmonary adenomas in nearly 100 per cent of the animals during the experimental period of 4 months. No difference in the number of animals developing local tumors was observed as compared with the controls. *p*-Dimethylaminoazobenzene fed in the diet also did not influence the number of local tumors elicited by methylcholanthrene.

Feeding of a diet containing *p*-dimethylaminoazobenzene alone augmented the number of mice bearing pulmonary adenomas to a significant degree over the spontaneous rate, while no liver damage could be detected under the conditions observed.

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