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Carcinogenic Action of Ethyl Urethane on Rats

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Recently Nettleship and Henshaw (6) observed a significant augmentation of the incidence of pulmonary adenomas of mice of the C3H strain treated by repeated injections of ethyl urethane. Henshaw and Meyer (1) confirmed this observation working with mice of the abc strain, which has a high spontaneous rate for tumors of this type. These authors have shown that the incidence of adenomas produced by the injection of urethane depends on the amount applied, and concluded that a single nonanesthetic dose is sufficient to produce some effect. Larsen and Heston (5) studied a number of narcotics with respect to their carcinogenic action. Only ethyl urethane and some other urethane derivatives were found to produce pulmonary adenomas in mice. We have found that ethyl urethane applied by injection or by mouth produced pulmonary adenomas in our noninbred strain of mice, which has a relatively low spontaneous incidence of lung adenomas (4). The action was very marked when the active substance was injected or given orally. Each author confirmed the high degree of specificity of urethane in producing only pulmonary adenomas, since no other type of tumor has been observed in the treated mice.

As pulmonary adenoma of mice is a special kind of relatively benign tumor, which very frequently occurs spontaneously, it seemed of particular interest to investigate the action of urethane on an animal species which does not develop this kind of neoplastic growth spontaneously. For this purpose rats seemed especially suited, as they seldom develop spontaneous lung tumors (2). The resistance of rat lung tissue against development of tumors has been shown also in experiments in which carcinogenic hydrocarbons have been injected intravenously. R. Jaffé (3) found only 2 pulmonary sarcomas in 90 rats injected intravenously with an oily solution of methylcholanthrene, while 30 per cent of the animals had tumors in some other site.

It therefore seemed of interest to investigate the carcinogenic action of urethane on rats. If the action of this substance is as specific as the results with mice seemed to indicate, it was hoped that some reaction of rat lungs would be produced which does not occur spontaneously.

MATERIALS AND METHODS

The rats used were from our own albino strain which shows a spontaneous tumor rate of about 5 per cent at the age of 1½ years. The spontaneous tumors observed are mostly malignant retroperitoneal or subcutaneous sarcomas, squamous epitheliomas of eyes and ears and a few cases of benign mammary adenomas. No spontaneous pulmonary tumors nor spontaneous hepatomas have ever been observed. All animals were 2 to 3 months old at the beginning of the experiments.

The urethane was administered by the oral route or injected intraperitoneally. In the first case the crystalline product was thoroughly mixed with the diet in an amount such that the food contained 0.15 per cent of urethane. The diet consisted of peanut presscake, ground corn, powdered milk, salt, and 2 per cent sesame oil, to which was added a concentrated preparation of vitamins A and D. The food was administered *ad libitum*. The animals gained weight on this diet containing urethane. It was continued until the rats died or were sacrificed. The last animals were killed after 15 months on the diet. In another series the urethane was injected; 1 cc. of a 10 per cent aqueous solution was administered to each animal by the intraperitoneal route. All the animals weighed about 100 gm. at the beginning of this experiment. The dose was sufficient to anesthetize them. The injection was repeated 30 times within 3 months. The early mortality was higher in this group than in the group that received the urethane with the diet.

Each rat that died or was sacrificed was autopsied and examined for the presence of tumors. Tissue of lung, heart, liver, kidney, and spleen was fixed in formol or Bouin solution and microscopic examination was performed in the usual manner.[1] It may be emphasized that the percentage of pulmonary adenomas reported may be a minimum number, because when the nodules were very small, it was possible to miss them in the cutting of the paraffin block. Only tumors

[1] We are indebted to Prof. R. Jaffé and Dr. J. A. O'Daly for the microscopic examination of the material. A detailed description will be published later.

that have been confirmed microscopically are reported in this paper.

RESULTS

In Table I a summary is given of the tumors observed in rats after treatment with urethane. As no tumor was found in animals that died within the first 3 months after the beginning of the treatment, we did not include them in the calculation. In the series fed a diet containing 0.15 per cent of urethane, 28 per cent developed pulmonary adenomas. If only those animals that survived one year or more are counted, the percentage of animals with pulmonary adenomas is 59. In the series receiving injections of urethane, the percentage of animals with lung adenomas was 7 in those that survived more than 3 months, and 8 in those surviving 1 year.

Microscopic lesions of a proliferative character have been observed in the lungs of a number of the treated animals. Metaplasia of the alveoli and proliferation of the bronchial epithelium have been found. In several cases these lesions existed together with real adenomas. The tumors were mostly multiple and varied greatly in size. Mostly they were about 1 mm. in diameter, in a few cases nodules of 5 mm. diameter have been found; in other cases the lesions were not visible macroscopically.

In addition to the lung tumors, we found a number of hepatomas, which must be regarded as elicited by the treatment with urethane. The percentage was less than that of the lung tumors; in the group of rats fed the diet containing 0.15 per cent of ethyl urethane only one case of an incipient and macroscopically invisible hepatoma has been observed, whereas the animals injected with that compound showed 27 per cent of hepatomas when the animals that survived more than 3 months are included. In all the cases of hepatomas observed in these animals the tumors were not visible with certainty macroscopically, although the tumor-bearing livers were of a dark color or showed some suspicious clear zones. Microscopically the tumors resembled those observed after the feeding of *p*-dimethylaminoazobenzene.

Also included in Table I are the animals of a series which had been treated identically with those receiving urethane injections, but in addition got a single intra-

TABLE I

Series no.	Treatment	Initial numbers of animals	Tumors found in animals dying at				
			3 mos.	6 mos.	9 mos.	12 mos.	15 mos.
71	Stock diet containing 0.15% urethane	57	10 negatives	8 negatives 1 bronch. prol.	8 negatives 2 bronch. prol. 2 metapl.	6 negatives 5 bronch. prol. 1 metapl. 2 pulm. aden.	2 bronch. prol. 2 metapl. 5 pulm. aden. 1 endothel. 1 sarcoma 1 hepatoma
66-B	30 Injections of 100 mgm. urethane each	28	10 negatives	2 negatives 1 hepatoma			7 negatives 3 hepatomas 1 pulm. aden. 1 sarcoma
66-A	30 Injections of 100 mgm. urethane each plus 1 i. v. injection of 2 mgm. methylcholanthrene	25	6 negatives	4 negatives 1 bronch. prol. 1 sarcoma	1 negative	1 negative 1 bronch. prol.	5 negatives 1 carc.+ bronch. prol. 2 hepatomas pulm. aden. 4 hepatomas

Tumors and proliferative pulmonary lesions observed in rats after treatment with ethyl urethane.

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Macroscopic aspect of lung of rat fed diet containing 0.15 per cent of ethyl urethane for 13 months. Two adenoma nodules are visible.

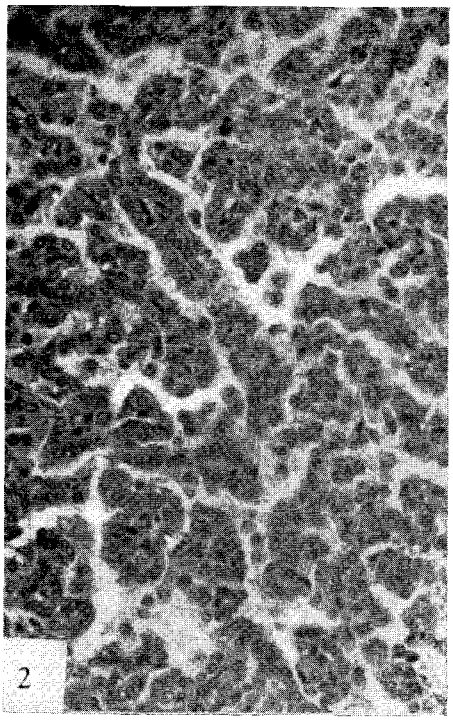
FIG. 2.—Microscopical aspect of pulmonary adenoma of rat fed diet containing 0.15 per cent of urethane for 12 months.

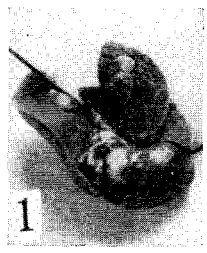
FIG. 3.—Microscopical aspect of lung adenoma in rat 10 months after having received 30 injections of 100 mgm. of urethane each.

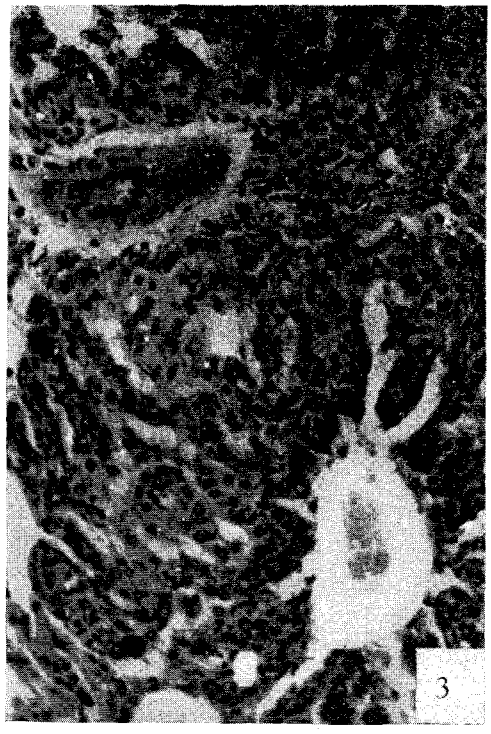
FIG. 4.—Epithelial metaplasia of alveolae in rat treated with urethane injections.

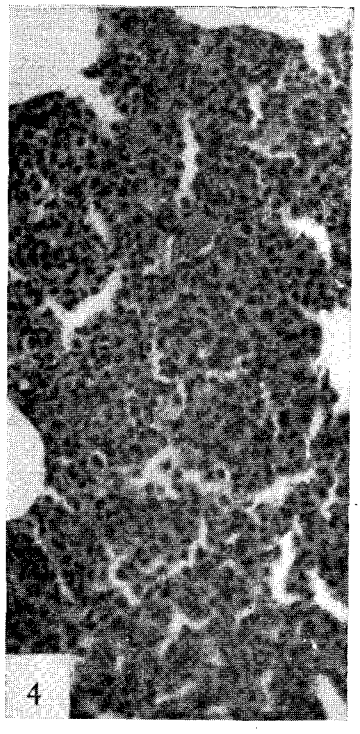
FIG. 5.—Hepatoma in rat treated with 30 injections of urethane and kil'ed after 15 months.

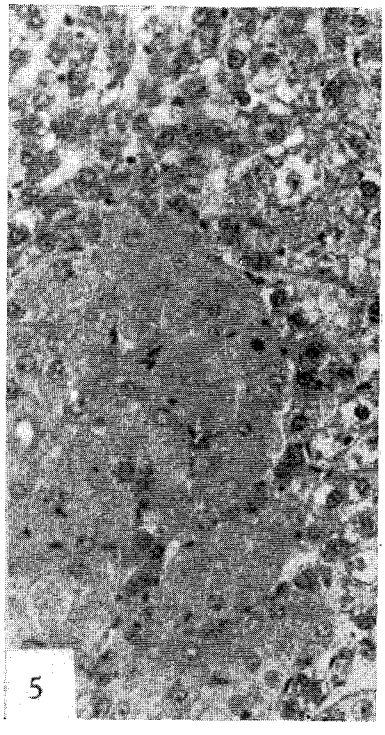
FIG. 6.—Hepatoma in rat treated with 30 injections of urethane and 1 injection of methylcholanthrene, killed 12 months after beginning of treatment.











FIGS. 1-6

venous injection of 2 mgm. of methylcholanthrene in olive oil. Nine per cent of this group showed pulmonary adenomas compared with 7 per cent of the series which received only urethane injections. The incidence of hepatomas was 27 per cent in both groups. The size of the liver tumors in the latter series was much greater. Three animals had macroscopically visible tumors of 3 to 30 mm. in diameter. The incidence of tumors in organs other than lungs or livers was 9 per cent in this group of animal that received methylcholanthrene. In previous experiments performed in this Institute with 60 rats of the same breed and kept under identical conditions, a single injection of the same amount of methylcholanthrene resulted in a 30 per cent incidence of tumors in various organs, mostly retroperitoneal sarcomas and squamous epitheliomas (3). In the present series only one case of a squamous epithelioma and one case of a retroperitoneal sarcoma has been observed in a effectual total of 19 animals. The number of animals in this series is not sufficient to draw definite conclusions as to whether the small number of tumors caused by methylcholanthrene is due to the simultaneous application of urethane. More experiments are under way to investigate the interaction of these two carcinogens in rats.

Of the total of 16 pulmonary adenomas observed in our rats, 13 were found in males and only 3 in females. At the beginning of the experiments the number of males and females was equal, but the females showed a higher premature mortality. Of the 35 rats which survived more than 1 year after the beginning of the experiments, 32 were males. It is therefore impossible to conclude whether a sex difference exists in respect to susceptibility to the production of pulmonary adenomas. The higher mortality of the females was chiefly caused by pregnancy and miscarriage.

Besides the tumors described, a few were found that must be regarded as spontaneous; 2 were retroperitoneal sarcomas, 1 with liver metastasis, and 1 endothelioma of the liver with lung metastasis. The incidence of these spontaneous tumors is about the same as usually observed with our animals.

DISCUSSION

The experiments described above demonstrated that urethane, administered by oral or parenteral routes, produces pulmonary adenomas in a high percentage of the treated rats. The effect is more accentuated when urethane is fed with the diet, in contrast to the observation in mice, where the parenteral route was more effective. Apparently urethane has the same action in mice and rats, producing lung adenomas in both species of animals. This is remarkable inasmuch as spontaneous pulmonary adenomas are very common in mice while they have not been observed in rats. Moreover the lungs of rats are very resistant to the action of other carcinogenic agents, as has been shown with methylcholanthrene (3). Nevertheless, under the action of prolonged application of urethane, this organ shows a marked reaction. Precancerous lesions can be observed such as metaplasia of the alveoli and proliferation of the bronchial epithelium. The first real lung adenomas were observed after 1 year. They may be barely visible to the unaided eye, while in some cases we found nodules of a diameter of 1 to 5 mm. Metastasis of these tumors have never been observed in our rats.

While the only reaction observed with urethane in mice is the production of pulmonary adenomas, in our urethane-treated rats some hepatomas were found also, which must be considered as produced by this substance. The series which received injections of urethane showed more hepatomas than the animals fed the substance with their diet. In the latter group only one case of incipient hepatoma was observed, whereas of the animals that had been injected with urethane, hepatomas developed in 27 per cent. Compared with *p*-dimethylaminoazobenzene, however, the action of urethane in producing hepatomas is weak. Administered with the diet at the level of 0.015 per cent, the latter substance produces 77 per cent of hepatomas in the treated animals of our strain within 15 months, while 0.15 per cent of urethane produced 1 single incipient hepatoma in 12 treated rats which survived more than 1 year on the diet. These animals must have ingested during the experimental period at least 7 gm. of urethane, whereas the rats injected with urethane received a total amount of 3 gm. Nevertheless, they developed more hepatomas than the first group. The interpretation may be that the time factor is more important than the dose for the production of hepatomas with urethane, while in respect to the development of pulmonary adenomas this is the opposite. The first group of animals received roughly half the amount of urethane during the first 3 months that the group treated by injection received. Only more extensive comparative studies can explain the different effects of feeding and injection of urethane in rats.

One group of rats was treated with injected urethane in the same manner as the group referred to above, but moreover was injected with 2 mgm. of methylcholanthrene in olive oil intravenously. The results observed in these animals with respect to the development of pulmonary adenomas and hepatomas did not differ significantly from that obtained in the series which did not receive the carcinogenic hydrocarbon. The hepa-

tomas observed in these animals were larger than those elicited by the treatment with injected urethane only. The same amount of methylcholanthrene administered to rats in an analogous way produced tumors (sarcomas and squamous epitheliomas) in 30 per cent of the treated animals (3), while in the present series only 9 per cent of the rats had these tumors. The question, whether the production of methylcholanthrene-elicited tumors is reduced by the simultaneous application of urethane, as these results seem to indicate, will be studied in further experiments.

The observation that urethane, injected or given by the oral route, is capable of producing pulmonary adenomas and hepatic tumors in rats should be taken as a warning against prolonged therapeutic use of this substance in human beings.

SUMMARY

One series of 57 rats was fed 0.15 per cent of ethyl urethane in the diet for 15 months. Fifty-nine per cent of the animals surviving more than 9 months developed pulmonary adenomas. Moreover, 1 case of an incipient hepatoma was found.

Twenty-eight rats were injected 30 times with 100 mgm. of urethane and 7 per cent of the animals surviving more than 9 months after the first injection developed pulmonary adenomas; 25 per cent developed hepatomas within 15 months.

Twenty-five rats received 1 intravenous injection of 2 mgm. of methylcholanthrene in olive oil and 30 injections of 100 mgm. in an aqueous solution of urethane intraperitoneally; 9 per cent of these animals had pulmonary adenomas and 27 per cent had hepatomas after 15 months.

Appendix

Histological Findings in Lungs and Livers of Rats Treated with Ethyl Urethane

Rudolf Jaffé, M.D.

A short description of the histological observations made in the lungs and livers of rats treated with urethane, as described in the foregoing paper, will be given.

The lung tumors found were mostly adenomas of the same type that occur in mice spontaneously or after treatment with carcinogenic hydrocarbons or urethane. They are well limited and consist of high epithelial cells, often glandular in arrangement. The cells are pale with large, well-formed and colored nuclei. Often transitions to other cell layers may be observed, which are more solid and consist of shorter cells. The cells are situated clearly intra-alveolars, but a connection with the branches of the bronchus has never been found. They are apparently derived from metaplastic alveolar epithelial cells.

A metaplasia of the alveolar cells without tumor formation can be observed frequently. These areas of metaplasia may be found in the neighborhood of atelectatic foci of various sizes or of vegetations of the connective tissue. As the metaplastic areas can be more or less of glandular-like aspect, it is sometimes difficult to decide whether the neoformation is a tumor or not.

Vegetations of bronchial epithelium are frequently observed; they may be situated either within or exterior to the bronchi. Vegetations with glandular appearance may be found surrounding larger bronchial branches. These formations probably do not deserve to be designated as tumors, although they may be quite tumor-like in their histological aspect. They are mostly found in combination with purulent bronchitis.

Infarcts were found frequently in the border zone of the lungs. They were always typical infarcts without the epithelial vegetations found in lungs of rabbits or rats injected intravenously with methylcholanthrene (3). The artery corresponding to the infarcted area showed an endoarteritic process, which was sometimes combined with a thrombus, but never with complete occlusion. The same kind of lesion of blood vessels without infarctation could be observed also, and infarcts without these lesions have been found. The blood vessels appeared dilated only in these cases.

The hepatomas found in the livers of the rats treated with urethane showed the same histological aspect as described by Opie (7) in rats fed *p*-dimethylaminoazo-

benzene. All the hepatomas observed in the present series were derived from hepatic cells and showed various aspects of solid and glandular forms. No tumors of the bile-duct epithelium similar to those in hepatomas induced by *p*-dimethylaminoazobenzene have been observed in these cases, although a few small bile-cell cysts have been found.

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