Carcinogenicity of Diethylnitrosamine in *Mystromys albicaudatus* (African White-Tailed Rat) (36573)

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In 1967 Hall *et al.* (1) suggested that *Mystromys albicaudatus* might serve as a useful new laboratory species for many types of investigations. Of interest was their finding that up to that time no neoplasms were seen in their colony involving several hundred animals which were up to 6 years old. One problem, in studies on carcinogenesis and on the mechanism of action of chemical carcinogens, has been that many of the rodent species currently used develop a certain incidence of spontaneous tumors in various tissues (2–5). This fact is a disadvantage in bioassay systems, particularly for the detection of weak carcinogens, for which lifetime studies are required to detect an effect, and thus control animals also exhibit tumors. It seemed that a species which in 1967 was reported to present with no spontaneous neoplastic disease was interesting if it were sensitive to typical chemical carcinogens.

Of all such carcinogens now known, the alkylnitrosamines appear the most versatile in the sense that every species so far tested responded positively (6, 7). With other agents, for various reasons such as specific biochemical activation or deactivation processes, there are greater differences in response as a function of species. Thus, for a preliminary investigation of the susceptibility of mystromys to a chemical carcinogen, diethylnitrosamine was the agent of choice.

The present paper reports our results indicating that the mystromys does develop tumors in the liver and in the forestomach with this carcinogen. However, higher dose levels are required for a longer period of time than with the currently used rodent species, such as rats, mice or hamsters.

Materials and Methods. Mystromys albicaudatus were obtained from the Rodent Production Section of the National Institutes of Health. As was discussed by Hall *et al.* (1) the average litter size is 2 to 3 after a gestation period of approximately 38 days. Seasonal variations in the productivity were observed. Thus, animals were procured over an extended period of time for these experiments. Usually, shipments of 4 to 6 mixed male and female weanlings were received. They were assigned to groups treated with diethylnitrosamine and controls, generally in a proportion of 2 to 1. For example, with a group of 6, 4 would be treated with carcinogen and 2 would not.

Diethylnitrosamine (or *N*-nitrosodiethylamine, Eastman Kodak Co., Rochester, NY) was dissolved in water to give concentrations of 200, 100 and 50 ppm, and administered thus in drinking water. Initially, the highest level appeared to be tolerated, but after some months on this dose a number of animals died. Thus, the remainder of these groups were switched to a lower dose of 50 ppm. Most of the mystromys received 100 ppm.

The animals were housed on Sanicell bedding in large plastic cages, usually in groups of 2 to 4, where they had access to a diet of Wayne Laboratory Blox and tap water, with or without carcinogen, *ad libitum*. Cages were changed and the entire equipment was sterilized once a week. Animals were carefully examined every day. When it appeared that survival was threatened they were necropsied, dissected, and tissues were fixed in Tellyesniczky's formol–alcohol solution. All ab-

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TABLE I. Tumor Induction in Male and Female *M. albicaudatus* with Several Dose Levels of Diethylnitrosamine (DENA) in Drinking Water.

Level of DENA in water (ppm)	Time on agent (weeks)	No. of animals		No. of animals[a] with									
						Bile duct				Gastric			
				Hepatoma		Carcinoma		Adenoma		Squamous cell carcinoma		Dysplasia	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
0 (control)	0–138	12	14	0	0	0	0	0	0	0	0	0	0
50	33	4	4	4	1	0	0	2	1	1	1[b]	1	3
100	25–40	12	15	7	5	3	3	1	2	1	1	4	6
200	22–42[c]	2	8	2	7	0	5	0	2	1	0	0	4

[a] Some animals had several of the lesions listed.

[b] Papilloma.

[c] One female and 3 males given a lower level of 50 ppm DENA for 13, and 7, 14 and 22 weeks, respectively.

normal tissues and select grossly normal tissues were subjected to conventional histologic processing, and the sections were stained with hematoxylin and eosin for microscopic study.

Results. The initial weight gain of mystromys at dose levels of 100 and 50 ppm diethylnitrosamine was similar to that seen in control animals. At the 200 ppm level growth rate was lower, indicating that this dose approximated the maximum tolerated dose, when the carcinogen is given for a limited time. After about 3 months animals in that group began losing weight and exhibited overt syndrome of toxicity. For this reason, the dose level of the remainder of the animals was lowered from 200 to 50 ppm, and the animals were held on this treatment for the remainder of their lives extending maximally to 42 weeks. With the 2 lower dose levels of diethylnitrosamine, the mystromys lived also for about 40 weeks.

The main lesions due to the treatment with diethylnitrosamine were in the stomach and in the liver (Table I). Males and females were equally affected. As reported (1) none of the control animals, some of which were held for up to 138 weeks, exhibited any tumors.

Pathology. Liver. The extent and nature of the liver lesions depended on the length of treatment. Changes were found both in the parenchymal cells and in the bile ducts. Enlarged hyperchromatic nuclei were noted in the parenchyma throughout the liver. In addition, hyperplastic areas, hyperplastic nodules, some with atypical regions, and hepatoma were seen. Most hepatoma were well differentiated. Approximately 30% of the hepatoma were mixed in that they contained both neoplastic hepatocytes and neoplastic bile ducts with transitional elements. The bile duct lesions consisted of a pronounced oval cell type and a tubular type proliferation, in which the latter often contained atypical cells characterized by their unusual form, basophilic cytoplasm and hyperchromatic nuclei. There was an accompanying fibrosis. Furthermore, bile duct adenoma (regular proliferating bile ducts with a defined fibrous capsule) and bile duct carcinomas were observed. In a number of cases cirrhosis was present. Table I lists the number of tumors developed. It was not an unusual finding that more than one hepatoma developed in the same animal. In addition, bile duct carcinomas, adenomas and hepatomas coexisted in a liver.

Stomach. As in most rodents, the stomach of the mystromys consists of a forestomach lined by a squamous epithelium, and a glandular stomach. The squamous epithelium in the forestomach exhibits papillary foldings of keratin, which increase in height with age. In a number of diethylnitroasamine-treated animals local hyperplasia of the squamous

epithelium was noted, with thickening of the basal cell layer. Atypical cells were observed in this layer. These lesions are denoted in Table I by the term dysplasia. In addition, in some animals squamous cell carcinomas were diagnosed. One animal bore 2 such carcinomas.

Discussion. In our laboratory male and female Fischer strain rats develop extensive hepatocellular carcinoma when given 40 ppm diethylnitrosamine in drinking water for only 10 weeks with a further 10-week holding period under control conditions. Similar fast cancer induction with this agent has been found in other laboratories with rats, mice and hamsters (6, 7). It seems quite clear even from the preliminary observations reported here that the mystromys responds much more slowly to higher dosages of this particular carcinogen than other rodent species currently used in experimental carcinogenesis studies. Considering the likelihood that this species would probably respond even more slowly with less potent carcinogens, or with agents which require specific metabolic activation (5, 8) it would seem that this species fails to meet the broad requirements for the bioassay of chemical carcinogens. This is true despite the fact that in our hands, as well as was previously demonstrated, animals up to 3 years of age had no spontaneous tumors, a desirable characteristic. Recently, a few spontaneous tumors were recorded even in this species, but most of them were found in animals older than 3 years in a species with an estimated life span of about 6 years (9). Biochemical studies on the nature and quantity of the enzyme systems, required for the activation of chemical carcinogens, may be useful to account for the lower response of the mystromys (10). The current results provide another example for the concept that the time required for tumor induction is proportional to the life-span of the species (2).

Of interest is the fact that in addition to liver tumors which included both hepatocellular carcinoma as well as bile duct tumors, the latter not induced often by diethylnitrosamine, mystromys also developed squamous cell carcinomas and dysplasia in the forestomach. This lesion is not usually apparent in other rodent species, although under some conditions gastric papillomas were seen in Chinese hamsters (7).

Summary. A newly introduced laboratory species, the rodent *Mystromys albicaudatus*, or African white-tailed rat, was subjected to oral administration in the drinking water of the carcinogen diethylnitrosamine at 3 dose levels. Male and female animals developed hepatoma, bile duct tumors and some squamous cell carcinomas in the forestomach with a latent period of 22 to 42 weeks. Control animals up to 138 weeks old had no tumors. This species can tolerate higher dose levels of diethylnitrosamine, but tumors developed after a longer latent period than in other laboratory rodent species used for bioassay of chemical carcinogens. This fact, and also the difficulty in reproduction of this species, due to litter size and length of gestation period, suggest that *Mystromys albicaudatus* is not as suitable for bioassay of carcinogens as currently used rodent species. Additional investigations with other agents are needed to fully support this conclusion.

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