TUMORS IN THE URINARY BLADDER OF A MONKEY: INDUCTION WITH 2-NITRONAPHTHALENE*1

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A rhesus monkey fed 2-nitronaphthalene for almost five years was found to have numerous papillomas of the urinary bladder when examined at autopsy. The chemical-induced neoplasms were composed of papillae of transitional epithelium which in some areas projected into the underlying lamina propria and submucosa. These epithelial cords showed metaplastic changes, but histopathology consistent with malignancy was not seen.

As part of a study designed to evaluate the rhesus monkey (Macaca mulatta) as a test animal for investigating the metabolism and carcinogenicity of certain bladder carcinogens, 2-nitronaphthalene was administered to three monkeys. This report is concerned with one monkey which survived 54 months of daily oral dosing with this compound.

The monkeys were young (2~3 kg), well-conditioned animals which had recently been imported from India. Before being assigned to the carcinogenesis study, the subjects had been infected with a laboratory strain of Plasmodium cynomolgi but had been cured with Chloroquine at least 12 weeks before being given the potential bladder carcinogen. The animals were maintained on a local modification of the Okatie ration, composition and use of which have been described elsewhere.4) 2-Nitronaphthalene, obtained through the courtesy of Dr. Julius Hyman, Fundamental Research Company, Berkeley, California, was purified with the gradient sublimation apparatus described by Case.1) The sublimed material was ground in a glass mortar and packed into hard gelatin capsules which were administered orally 6 days per week by means of a modified stomach tube. The daily dose of 242 mg/kg body weight was administered in two divided doses, one at midmorning and one at midafternoon.

Chromatographic methods utilized for identification of certain urinary metabolites were essentially those previously described.2)

Although two of the three monkeys were sacrificed after only one month of treatment, urine collected from these animals was found to contain 2-amino-1-naphthyl sulfate and 2-acetamido-6-naphthyl glucuronide. These two metabolites were also identified in the urine specimens collected from monkeys which were given 2-naphthylamine,2) indicating that 2-nitronaphthalene was reduced in this species. Although the possibility of reduction of the orally administered 2-nitronaphthalene by intestinal bacteria must be considered, the urine samples collected from monkeys treated with 2-nitronaphthalene contained compounds not found when 2-naphthylamine was administered. Also, although these latter metabolites have not yet been identified, preliminary work suggests the integrity of the nitro group.

The monkey which was treated for 54 months was killed with an overdose of sodium pentobarbital and subjected to necropsy examination. At autopsy, the genito-urinary tract was
removed en bloc and, after ligation of the urethra, approximately 10 ml of Zenker-Formalin fixative was instilled into the urinary bladder via the ureters. After fixing and washing, tissue specimens were processed through paraffin, sectioned, and stained with Hematoxylin and Eosin.

**Gross and Microscopic Findings**

Terminal weight of the female monkey was 5.7 kg. No gross pathologic lesions were noted in organs other than the urinary bladder; it contained one papilloma (0.5 × 2 mm) in the trigone area and two papillomas (0.5 × 2 mm) and one fungating tumor, 4 mm in diameter, in the left lateral wall (cf. Photo 1).

**Histological Examination**

There were multiple, irregular, papillary tumors composed of papillae of transitional epithelium. The transitional epithelium over the papillae was thickened, but it retained good differentiation. Papillary foldings resulted in transitional epithelial cords beneath the superficial layer. In some areas the transitional epithelium projected into the underlying lamina propria and submucosa. These ingrowths of transitional epithelium usually show metaplastic change into glands lined with well-differentiated columnar cells. Copious acidophilic secretion was found in the gland lumens. There was no evidence of neoplastic invasion. Diagnosis: Papilloma (cf. Photo 2).

Although Treon and Cleveland reported single doses of 2-nitronaphthalene to two rhesus monkeys they did not report the identity of any of the urinary metabolites. Results of the present preliminary investigation indicate that 2-nitronaphthalene is reduced in the rhesus monkey and that one or more of the urinary metabolites has the potential for inducing neoplastic changes in the transitional epithelium of the urinary bladder. Although carcinoma was not observed in the one animal described, malignant tumors similar to those induced in monkeys with 2-naphthylamine might be anticipated if a large number of monkeys were at risk.

Although 2-nitronaphthalene has not been reported as carcinogenic for man, the findings reported here would suggest that those persons who handle this compound should exercise the same precautions as for 2-naphthylamine and 4-nitrobiphenyl.

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**REFERENCES**


**EXPLANATION OF PLATE XII**

Photo 1. Longitudinal section of fixed urinary bladder showing papillomas (see arrows).

Photo 2. A section of the bladder epithelium showing a portion of one of the papillomas at the left transition and into normal mucosa to the right in photograph. The neoplasm contains transitional epithelial cords beneath the lamina propria. In its deeper parts some of these transitional cells show formation of glands containing acidophilic secretion. Hematoxylin and Eosin stain. ×40.