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Ultrastructure of Tumors Induced in the Rat Urinary Bladder by Nitrosomethyldodecylamine

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Summary. N-Nitroso-N-methyl-n-dodecylamine (NMDA) is a powerful carcinogen in the rat and the Syrian golden hamster. In both species the urinary bladder is the main target organ. We studied the ultrastructure of these bladder tumors in the Fischer rat in some detail, since this compound provides an interesting model for carcinogenesis in the urinary bladder. We found that the proliferating basal layers of the transitional cell carcinomas were undergoing squamous metaplasia, which indicates that squamous carcinomas in the organ may arise from pre-existing transitional cell tumors.

Key words: Nitrosomethyldodecylamine – Bladder carcinogenesis

Introduction

Dimethyldodecylamine has been used as an anti-suckering agent for tobacco plants, and may well be present as a contaminant of tobacco products. Under mildly acid conditions, N-nitroso-N-methyl-n-dodecylamine (NMDA) can be formed by reaction with nitrous acid (Lijinsky et al. 1972). This compound is a powerful carcinogen in rats (Lijinsky and Taylor 1975, 1978), and Syrian golden hamsters (Althoff and Lijinsky 1977), with (in both species) the urinary bladder as the main target organ. In the rat, the response is very uniform, with transitional cell carcinomas developing at an incidence of 100% (Lijinsky and Taylor 1975, 1978), whereas, in the hamster, a variety of other organs are affected: in the urinary bladder, tumors of a number of different types are seen (Althoff and Lijinsky 1977). This compound provides an interesting model for carcinogenesis in the urinary bladder, and we report here the ultrastructure of NMDA-induced tumors in this organ in the Fischer rat in detail.

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Materials and Methods

Twelve male F344 rats were maintained in an animal barrier facility, four per cage. At 11 weeks, they were divided into two groups. One group of eight rats received 12 mg NMDA in 0.2 ml olive oil by gavage twice weekly for 28 weeks. A control group of 4 rats was treated with olive oil alone. The animals were kept until, at 55 weeks after the start of treatment, symptoms of bladder tumors were observed (bleeding, loss of weight). Under pentobarbital-anesthesia (Diabutal, Diamond Lab., Des Moines, IA), the rats were fixed in situ by vascular perfusion with 2% cacodylate-buffered glutaraldehyde (pH 7.4). Urine was withdrawn from the urinary bladder with a syringe and the organ filled with the fixative. The bladders were then excised and macroscopically visible tumors were cut into 1 mm pieces. The samples were fixed for an additional 2 h in glutaraldehyde and postfixed 1 h in 1% cacodylate-buffered osmium tetroxide. After dehydration in graded ethanols, the samples were embedded in Epon 812 (Ladd Research, Inc., Burlington, VT.). Sections were cut on an LKB III Ultramicrotome. Semithin sections for light microscopy were stained with toluidine blue. Ultrathin sections were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. They were examined in a Philips 201C electron microscope; electron micrographs being taken at 60 Kv.

The remainder of the bladders, including the tumors, and the lungs, livers, and kidneys were processed for routine histology from parafin-embedded hematoxylin/eosin stained material.

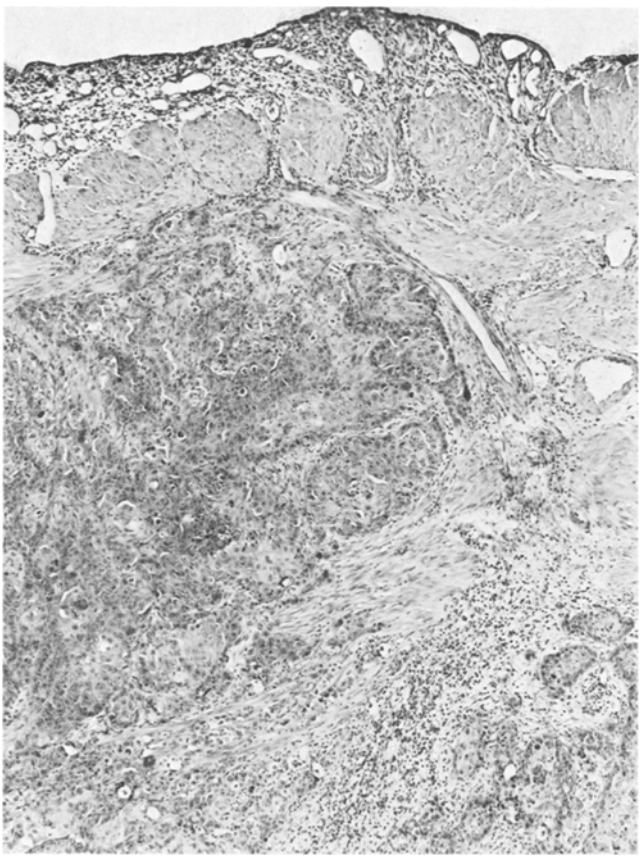

Fig. 1. Histology of a typical NMDA-induced transitional cell carcinoma: the tumor cells are arranged in nest-like patterns and invade the muscle layer of the bladder wall. ×40

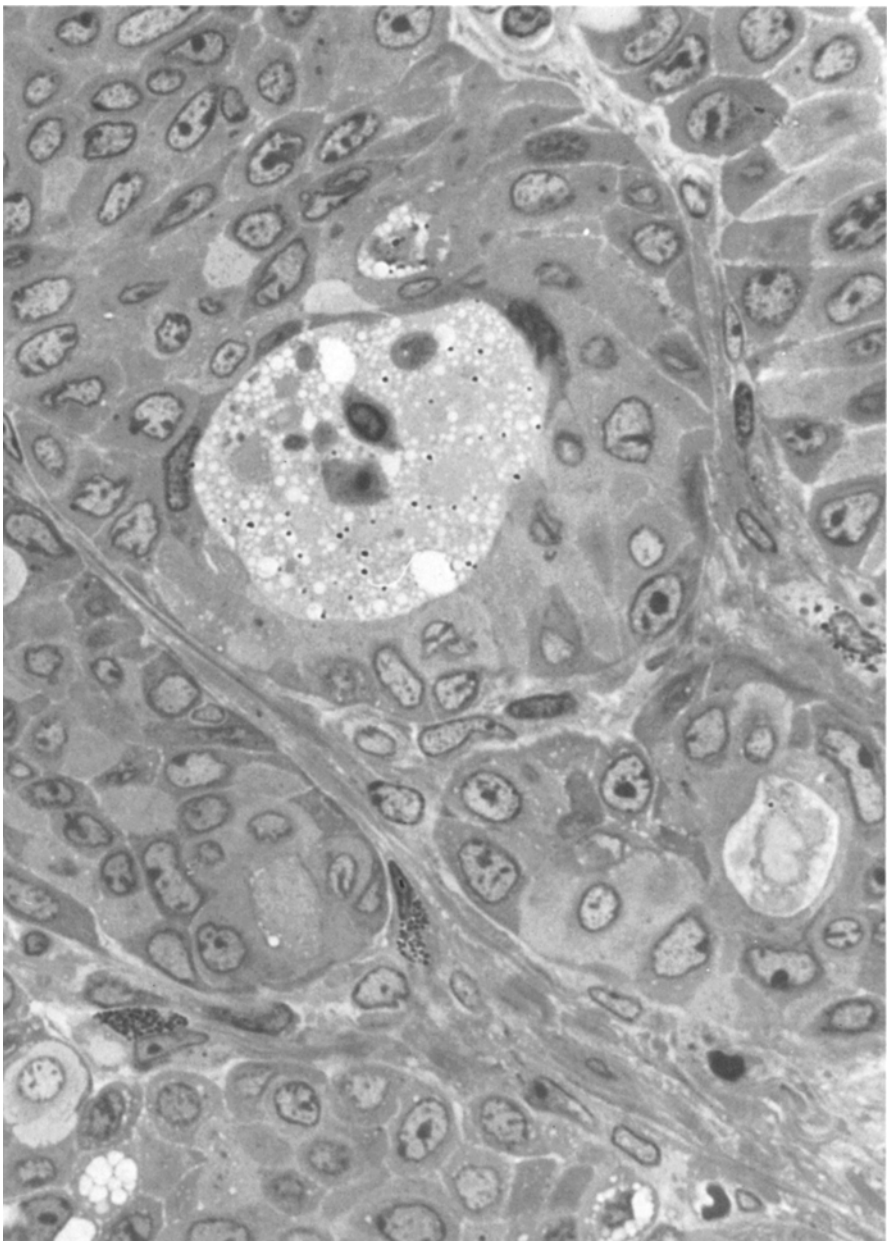

Fig. 2. High power light micrograph exemplifying cyst-like spaces filled with fluid. × 1,000

Results

All of the NMDA-treated rats had tumors of the urinary bladder which were diagnosed as transitional cell carcinomas by light microscopy (Fig. 1). The neoplasms were composed of solid nests and cords of epithelial cells which invaded

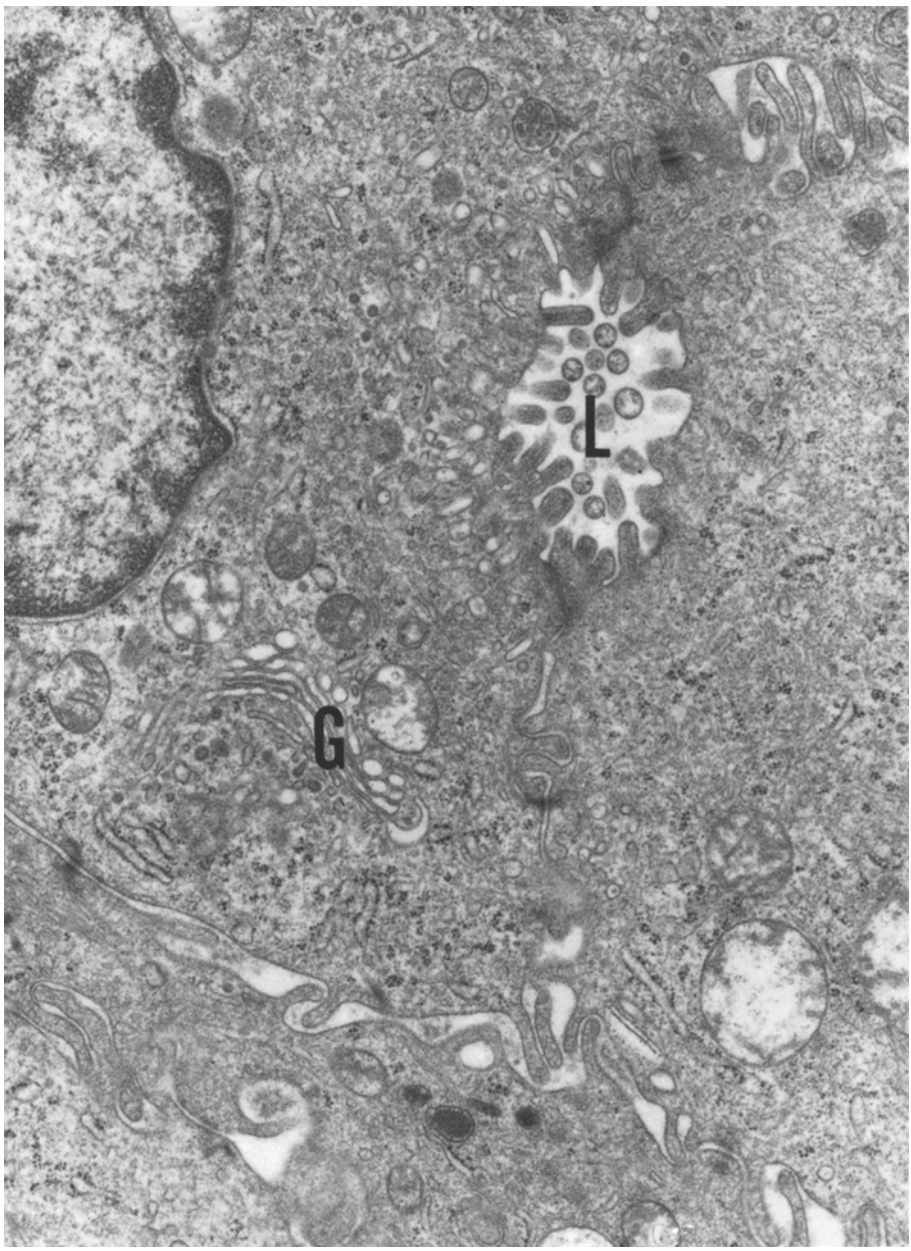

Fig. 3. Electron micrograph demonstrating an intraepithelial lumen (*L*) lined by microvilli. The cell on the left has abundent surface vesicles and a well-developed Golgi apparatus (*G*); note also tight junctions at lumen and desmosomes more peripherally. ×20,000

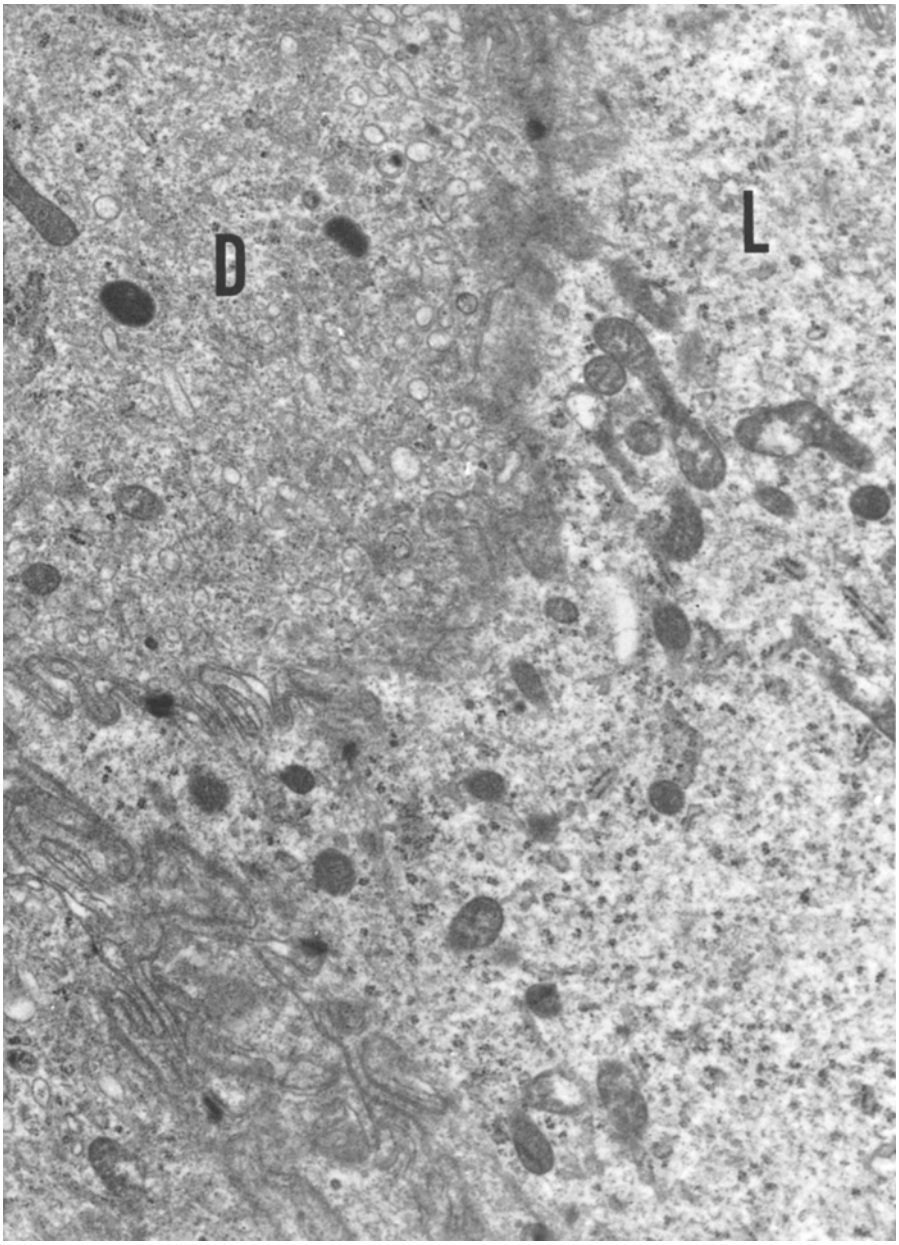

Fig. 4. Electron micrograph exemplifying light (*L*) and dark (*D*) cells. ×9,000

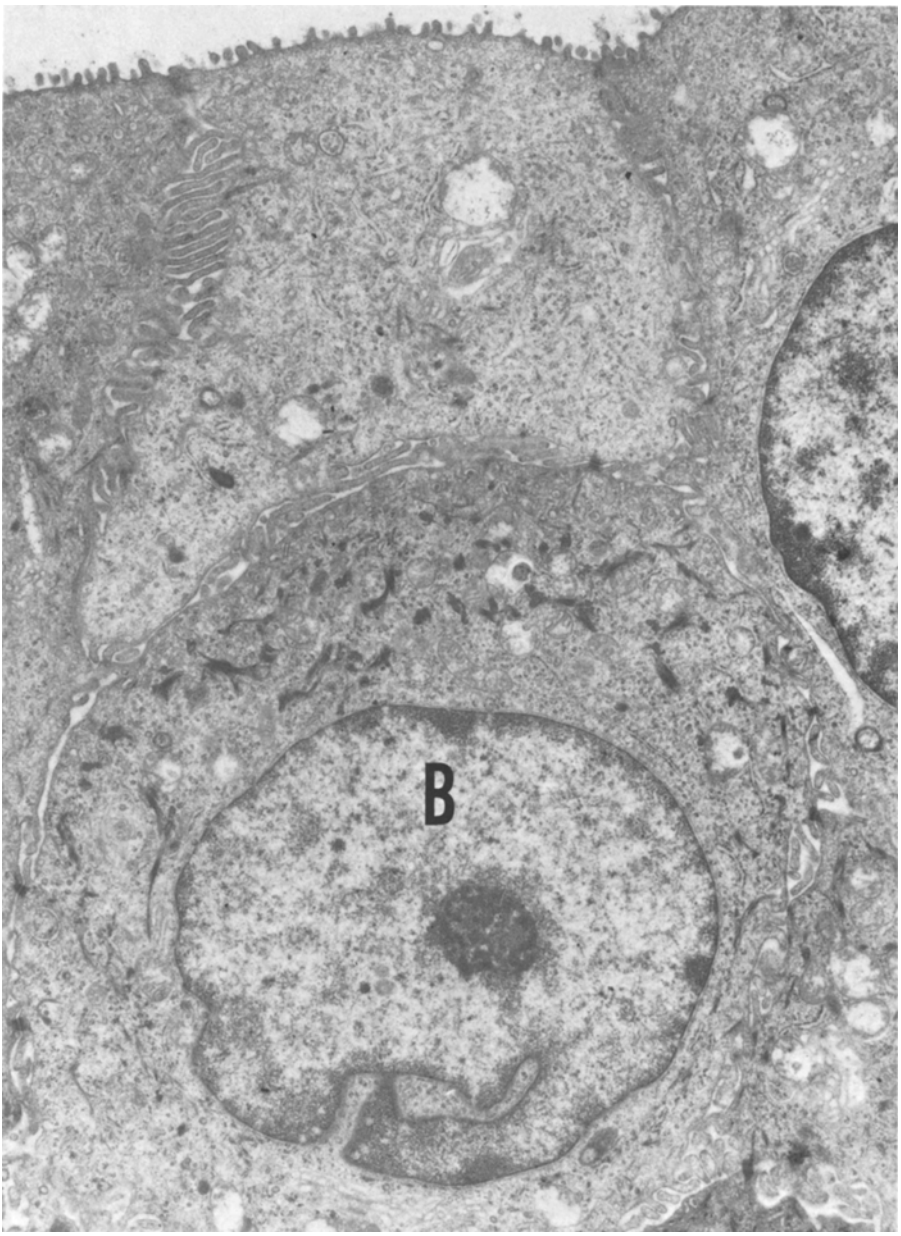

Fig. 5. Electron micrograph exemplifying differences in the differentiation pattern of cells located at a lumen and in the basal layer (*B*). ×9,200

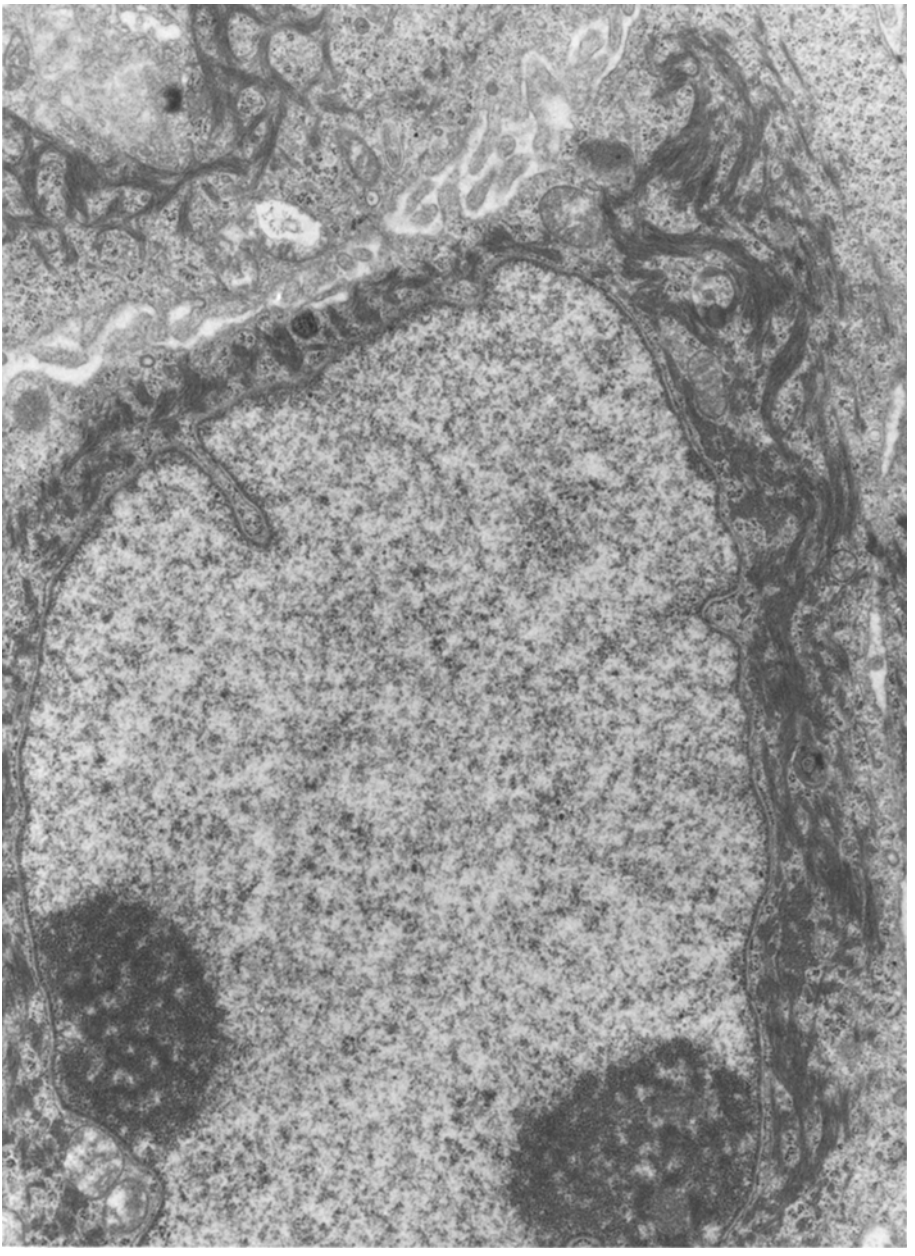

Fig. 6. Tumor cell from basal layer: bundles of tonofilaments are prominent; note also sparse nuclear heterochromatin and two nucleoli. ×16,000

the muscle layer of the bladder wall (Fig. 1). The centers of such cell nests were often occupied by a cyst filled with fluid and cell debris (Fig. 2). Individual cell nests and cords were surrounded by basement membranes and little connective tissue. The tumor cells differed widely in their level of differentiation. Cells which enclosed central spaces, as well as cells close to the lumen of the urinary bladder, had well-developed microvilli (Fig. 3) covered by a glycocalyx. The surface areas of their cytoplasm possessed abundant small vesicles (Fig. 3). A well-developed Golgi apparatus, short tubules of rough endoplasmic reticulum, and a moderate number of mitochondria were generally found in these cells (Fig. 3). At their luminal edge, the interdigitating borders of adjacent cells were linked by tight junctions while their more peripheral parts were connected by desmosomes (Fig. 3). Differences in the density of the cytoplasm were occasionally noticeable among individual cells (Fig. 4). These cells, which appeared dense, possessed more cytoplasmic organelles (e.g., vesicles, ribosomes) and they also had filaments which were lacking in the less dense cells. In the layers of tumor cells located close to the basement membrane, the pattern of differentiation was quite different. Their major cytoplasmic organelles were ribosomes and polyribosomes while the endoplasmic reticulum was scanty. Delicate tonofilaments which formed prominent bundles (Figs. 5, 6) were found in many of the basally located cells. The nuclei of such cells appeared pale (Figs. 5, 6) because they had little condensed heterochromatin. The nucleoli were prominent (Fig. 6) and often multiple. Mitotic cells were mainly located in the basal layers of cells. Many of the mitotic cells contained bundles of tonofilaments.

Discussion

Our study reveals pronounced differences in the differentiation of the cells of NMDA-induced bladder tumors. While cells located at epithelial surfaces exhibited many features of normal transitional epithelium, the cells constituting the basal tumor layers had characteristics of squamous cells. Among the properties which the neoplastic cells shared with the normal transitional epithelium were cytoplasmic surface vesicles, thight junctions between the surface parts of neighboring cells and desmosomes between their more basal parts as well as the presence of a well-developed Golgi apparatus (Leeson 1966; Petra and Amon 1966; Reale et al. 1963; Battifora et al. 1964). In contrast, microvilli and a glycocalyx are not found in the normal transitional epithelium of the urinary bladder. These specialized surface structures were first described in this organ by Hicks et al (1969, 1974, 1975, 1976), and they are considered to be a morphological marker for neoplastic transformation in the urothelium of man and other mammals (Hicks and Wakefield 1976).

In general, the basal portion of the tumors appeared less well-differentiated than did their luminal counterparts. Basal cells at various stages of squamous metaplasia, manifested by different amounts of tonofilaments and filament bundles, occupied these areas of the neoplasms. Their low nuclear/cytoplasmic ratio, sparse heterochromatin and scanty cytoplasmic organelles were pathognomonic for actively proliferating cells. Accordingly, mitotic cells were numerous

in these basal layers of the tumors. Our results indicate that, in this model system, squamous tumors may arise from transitional carcinomas. This interpretation is in agreement with the findings of Hicks (1968) who considered chemically-induced squamous metaplasia of the urothelium to be a manifestation of a pre-existant keratin-synthesizing mechanism. On the other hand, the squamous areas in the basal tumor parts could also be interpreted as squamous metaplasia of transitional epithelium. This in turn would suggest that focal transformations of the bladder epithelium were induced which become sometimes manifest as squamous rather than transitional epithelium "phenotype". A final clarification of the sequence of events in this experimental system would require to study the mode of tumor development by sequential analysis.

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