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TOXICOLOGY AND APPLIED PHARMACOLOGY 90, 359–376 (1987)

Effects of Acute and Chronic Dimethylamine Exposure on the Nasal Mucociliary Apparatus of F-344 Rats[1]

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Received January 15, 1987; accepted May 15, 1987

Effects of Acute and Chronic Dimethylamine Exposure on the Nasal Mucociliary Apparatus of F-344 Rats. GROSS, E. A., PATTERSON, D. L., AND MORGAN, K. T. (1987). *Toxicol. Appl. Pharmacol.* **90**, 359–376. Dimethylamine (DMA) is a highly water soluble gas with many industrial applications. Male F-344 rats were exposed to 175 ppm DMA 6 hr per day for 1, 2, 4, or 9 days or 2 years. Gross changes in nasal structure were recorded, effects of DMA on the mucociliary apparatus were assessed using video analysis, and tissues were evaluated for histopathology. *In vitro* nasal mucociliary flow patterns, mucus flow rates, and ciliary activity were studied and recorded for video motion analysis. There were distinct and generally consistent differences in the shape of the naso-, maxillo-, and ethmoid turbinates between young and old animals. Acute and chronic DMA exposures resulted in erosion of the anterior margins of the naso- and maxilloturbinates and fenestration of the adjacent septum. Ciliastasis and mucostasis were observed only on the anteromedial aspect of the maxilloturbinate. In the chronically exposed rats, mucociliary activity was present in areas adjacent to erosions of the turbinates and septum. Abnormal mucus flow patterns, including altered or reversed direction of flow and "whirlpool-like" formation, were observed in all treated rats, but were more severe following chronic exposure. There was a good correlation between the distribution of responses as assessed by histopathology and abnormal mucociliary function at all time points. In conclusion, the mucociliary apparatus continues to function in the nasal passages of rats having localized destruction of nasal epithelium, induced by DMA exposure, and this clearance system responds to alterations of nasal structure by modification of mucus flow patterns.

Dimethylamine (DMA) is a flammable colorless gas which is used in a variety of industrial applications and consumer products including agricultural fungicides, acid gas absorbants, flotation agents, soaps and cleaning products, and the production of a number of pharmaceutical products (*New Drugs,* 1966; Braker and Mossman, 1971). Human exposure to dimethylamine occurs by mainly skin contact or direct inhalation. It is considered extremely toxic, receiving toxicity scores of 5, 4, and 4 for inhalation, eye contact, and skin irritation, respectively, using a 1 to 5 scale (5 being the most toxic) reported by Sunshine (1969). However, DMA is considered to have very good warning properties with an odor threshold of approximately 0.60 ppm (Bond *et al.,* 1972). The current threshold limit value (TLV) for DMA was set at 10 ppm to protect against respiratory tract and ocular irritation (*Documentation of the Threshold Limit Values,* 1971).

Fischer-344 rats exposed to DMA at the LC50 concentration of 4540 ppm developed mild to severe ulceration and necrosis of the nasal turbinates and trachea (Steinhagen *et*

[1] This work was presented in part at the Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics, August 1983.

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0041-008X/87 $3.00

al., 1982). Rats exposed to 175 or 250 ppm DMA (6 hr/day, 5 days) or 500 ppm DMA (6 hr/day, 3 days) developed ulcerative rhinitis, severe congestion, and squamous metaplasia in the respiratory tract with the most severe lesions occurring in the anterior sections of the nasal cavity (W. H. Steinhagen and J. A. Swenberg, unpublished results). Fischer-344 rats and B6C3F1 mice exposed to DMA at concentrations of 10, 50, and 175 ppm for 6 or 12 months showed concentration-related lesions in the nasal passages, including focal destruction of the anterior nasoturbinate and nasal septum, local inflammation and focal squamous metaplasia of the respiratory epithelium as well as loss of sensory cells and olfactory nerves, and respiratory metaplasia of the olfactory region (Buckley *et al.*, 1985). It was suggested in the latter study that squamous metaplasia of the respiratory epithelium and respiratory metaplasia of the olfactory epithelium may represent adaptive or defensive responses to DMA exposure.

The respiratory epithelium of the nasal passages is characterized by the presence of the mucociliary apparatus, which is known to provide some protection against inhaled particles and may function as a defense against inhaled water-soluble gases (Morgan *et al.*, 1984; Patterson *et al.*, 1986). Postmortem assessment of mucociliary function was found to provide a more sensitive indicator of toxicity than histopathology in rats exposed to another gaseous irritant, formaldehyde (Morgan *et al.*, 1986). This concept has been investigated primarily in relatively young animals and following relatively short exposure periods (up to 10 days). Information on mucociliary function in the rat as it ages and responses to life-time exposure to irritants is lacking in the literature. The purpose of the present study was to examine the effects of DMA on the nasal mucociliary apparatus of the F-344 rat following acute and chronic exposure. In addition to this, the normal mucociliary apparatus of the F-344 rat at 2 years of age was examined for the first time. Changes in nasal structure that were discovered during the course of the study and thought to be associated with aging or with DMA exposure were also examined. Histopathology of DMA-induced lesions detected in the nasal mucosa which had not been reported previously are also described.

METHODS

Animals. Groups of six male Fischer-344 rats (CDF (F-344)/CrlBR; Charles River Breeding Laboratories, Kingston, NY) were exposed to DMA at a concentration of 175 ppm, 6 hr per day for 1, 2, 4, or 9 days or 2 years. The 2 year animals were part of a CIIT sponsored bioassay (Buckley *et al.*, 1984). Six animals were examined at each concentration. Two groups of control animals were used in this study, 12 acute control animals (6 weeks old) and 6 chronic control animals (2 years old) derived from the DMA bioassay. The terminal body weights of the animals were 241.67 ± 4.99, 238.17 ± 4.29, 225.33 ± 1.84, 221.08 ± 3.17, and 346.50 ± 9.82 for animals exposed for 1, 2, 4, or 9 days or 2 years, respectively, and 228.25 ± 4.20 and 355.83 ± 11.92 for the acute and chronic control groups (values are expressed as means in grams ± SE). Animals for the acute study were housed in polycarbonate cages on wire racks, over heat-treated hardwood bedding. They were transferred to stainless steel exposure cages which were placed on top of the stainless steel cage racks in the exposure chambers during the exposure periods. Chronic study animals were housed individually in hanging stainless steel wire-mesh cages in the exposure chambers. Pretesting of the chamber for concentration calibration indicated that the concentration of DMA was stable and was found to be within 10% of the nominal concentration at all points within the chamber (W. H. Steinhagen, personal communication). During periods of nonexposure, the animals were provided with food (NIH-07 open formula diet, Ziegler Brothers, Gardners, PA) analyzed for selected contaminants (Lancaster Labs, Lancaster, PA) and tap water *ad libitum*. The chambers housing the animals were maintained on a 12-hour light/dark cycle. All animals used in the acute phase of this study had negative viral titers in a standard murine virus antibody determination (Microbiological Associates Inc.). Sera from selected animals in the chronic study chambers (none were from animals used in this study) were negative with the exception of a few (3/12 controls, 1/12 175 ppm DMA) positive results for rat corona virus.

Exposures. The method for test atmosphere generation and analysis was described in detail by Buckley *et al.* (1985) and is outlined briefly here. Dimethylamine (99.97% pure) was obtained from Air Products and Chemicals, Inc. (Fogelsville, PA). Rats were exposed and housed in 8-m^3 glass and stainless steel chambers with a total airflow of 2200 liters/min. The analytical concen-

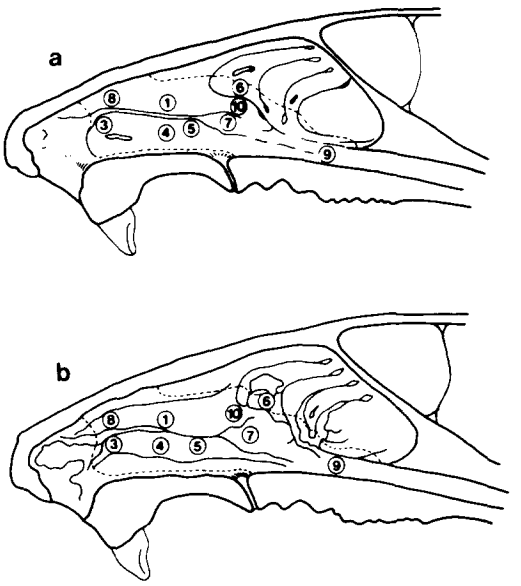

FIG. 1. Diagrams of young (10 week) (a) and old (2 year) (b) nasal cavities showing the location of the areas where video recordings were taken for mucus flow rate analyses. The numbering system is the same as that used in previous work (Morgan *et al.* 1984).

tration of DMA, expressed as a time-weighted average over the entire 24 months of exposure, was 175.1 ± 1.9 (ppm ± SD). Temperature and relative humidity were 71.5 ± 0.9°F and 55.1 ± 6.1%, respectively. The temperature and relative humidity in the control chamber were 71.7 ± 0.8°F and 50.8 ± 4.8%. Pure DMA was metered from the cylinder through flow meters into the supply airstream. Test atmosphere analysis was performed at 15-min intervals by infrared spectrometry.

Mucociliary studies and histopathology. Rats were killed by decapitation within 1 hr of the end of the exposure period. Following the procedure described by Morgan *et al.* (1984), the nasal cavity was opened by rapid dissection. The dissected tissues were placed in a 145-ml stainless steel and glass observation chamber which was maintained at 36–38°C. Humidified air was passed through the chamber at a rate of 1 liter/min during the entire observation period. The surface of the nasal mucosa was lighted with a fiber-optic light on a microscope fitted with long-working distance objectives. The mucosal surface was examined and mucus flow patterns, ciliary activity, features of nasal structure, and abnormalities of mucociliary function were recorded manually on maps of the nasal passages. Video recordings were made at 12 preselected locations (Figs. 1a and 1b) for subsequent video analysis. Video recordings to be used for the determination of mucus flow rates were made within 20 min after death because previous work has shown that mucus flow remains fairly constant for at least 20 min after death in untreated F-344 rats (Morgan *et al.*, 1984). Maps of the rat nasal passages were used to record times of the various recordings as well as observations on the nature and extent of defects in mucociliary function. Ciliastasis was defined as absence of visible ciliary beat. Mucus flow rates were determined from video recordings by timing the travel of particles in the mucus epiphase over a calibrated distance on the monitor. The statistical significance of flow rate data was assessed by one-way analysis of variance. Dunnett's multiple range test with a pooled estimate of variance was used to compare each treatment group to the appropriate control group. A nominal statistical significance level of 0.05 was selected. Because there was a difference between flow rates of acute and chronic control animals, data from treated animals were compared with the data of the respective controls. A computer software package (RS/1, BBN Research System, Cambridge, MA) was used for the statistical analyses. Following mucus flow studies, tissues were fixed in 10% neutral buffered formalin and processed to paraffin, and 6-μm sections were cut, stained with hematoxylin and eosin, and examined by light microscopy.

RESULTS

Gross Appearance of the Nasal Passages (Control Rats)

Gross structure of the nasal passages of young and old rats are shown for comparative purposes in Figs. 2a and 2b. Age-related changes in the nasal passages included a more intense greenish yellow coloration of the olfactory epithelium and altered shape of the turbinates. The characteristic smooth outlines of the turbinates of young rats (Fig. 2a) were replaced by irregularities of the margins of the naso- and maxilloturbinates, and a distinct downward or hook-like curvature of anterodorsal extension of the median ethmoid endoturbinates (Fig. 2b). In young rats, the lateral wall is visible through the space between the naso- and maxilloturbinates, while this is not possible in older animals due to overlapping of the turbinates. These differences are readily appreciated on comparison of Fig. 2a with Fig. 2b.

Mucociliary Function

Controls. Mucus flow patterns and flow rates (Table 1) from young rats were consis-

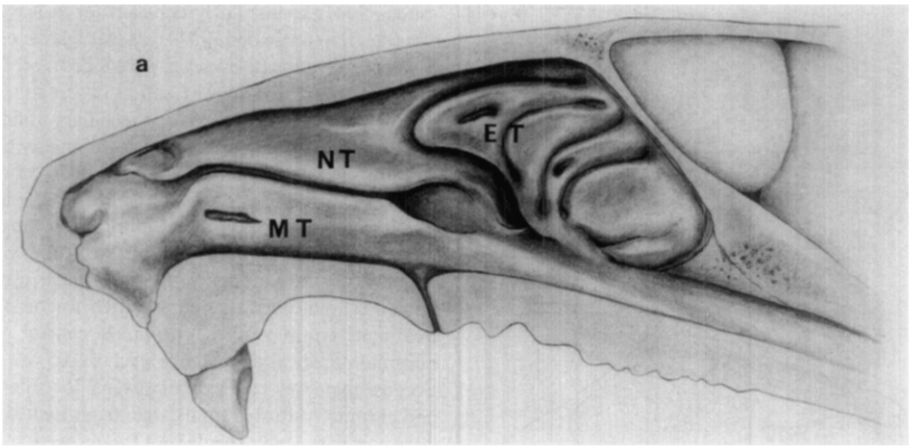

FIG. 2a. Drawing of a young (10 week) rat nose which has been opened longitudinally to show the structure of the nasal turbinates for comparison with an old rat in Fig. 2b. NT, nasoturbinate; MT, maxilloturbinate; ET, ethmoid turbinates.

tent with data from previous studies (Morgan *et al.*, 1984). In older rats (Fig. 3), mucus flow patterns resembled those seen in young animals. However, in old rats the nasal mucus appeared to be more abundant. Mucus flow rates were slower than those of young animals in all areas examined (Table 1), but were statistically significant only in areas 4 and 9 (Fig. 1.).

DMA-exposed animals: acute study. Exposure to DMA resulted in erosion of the margin of the naso- and maxilloturbinates (Fig.

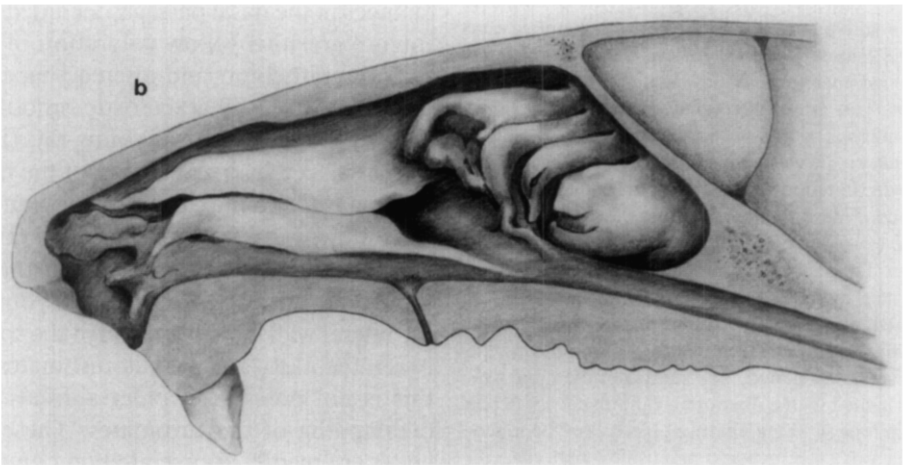

FIG. 2b. Drawing of old (2 year) rat nose which has been opened longitudinally to show the structure of the nasal turbinates for comparison with the young rat in Fig. 2a. Note the increase in size of the posterior naso- and maxilloturbinates and the overlap of these structures. Also note the change in shape of the ethmoid turbinates. The anterodorsal scroll of the ethmoid turbinate is curved ventrally to produce a "hook-like" profile. The olfactory area of the ethmoid turbinates in older animals also appeared more darkly pigmented than that of the young rats.

TABLE 1

MUCUS FLOW RATES IN SELECTED AREAS OF THE RAT NOSE FOLLOWING TREATMENTS WITH DMA (175 ppm)

Region of the nose[a]	1	4	5	6	7	8	9	10
Acute controls	5.95[b] (1.57)[c]	2.86 (1.99)	3.45 (2.28)	2.22 (2.08)	11.45 (7.41)	2.53 (1.69)	6.29 (3.12)	9.70 (4.46)
1 Day	4.61 (2.75)	1.31 (1.41)	2.11 (1.09)	0.94 (0.71)	11.30 (4.33)	ND[d]	6.02 (3.00)	ND
2 Days	2.32* (2.38)	1.71 (1.93)	1.68 (0.97)	1.41 (0.10)	7.29 (1.69)	ND	7.59 (1.51)	7.71 (2.33)
4 Days	4.76 (2.15)	1.82 (1.27)	3.92 (1.29)	ND	8.17 (2.41)	1.50 (1.41)	8.33 (3.41)	12.97 (2.28)
9 Days	3.09 (3.66)	0.70 (0.49)	4.88 (2.00)	ND	6.55 (8.16)	ND	7.66 (3.94)	14.82 (8.05)
Chronic controls	2.31 (1.79)	0.49* (0.01)	2.26 (0.65)	0.97 (0.23)	5.11 (4.95)	ND	2.39* (1.81)	3.43 (2.87)
2 Years	1.40 (1.32)	1.63 (1.89)	4.17 (2.86)	1.60 (0.62)	6.23 (3.35)	ND	6.37 (6.72)	3.32 (0.13)

[a] See Fig. 1.
[b] Mean (mm/min).
[c] (Standard deviation).
[d] ND, measurements could not be done because of poor recordings or paucity of particles.
* Significantly different from acute control mean at $p < 0.05$.

4) at all time points examined and fenestration of the adjacent septum. Altered mucus flow patterns were seen after only 1 day of exposure and the severity was unchanged following multiple exposures. These changes included early reversal of flow on the posterior maxilloturbinate and rotational flow on the posterior lateral wall just anterior to the nasopharynx (Fig. 4). Abnormalities of mucus flow patterns and the areas of ciliastasis (absence of ciliary activity; Fig. 4) were more extensive with increased exposure time. In

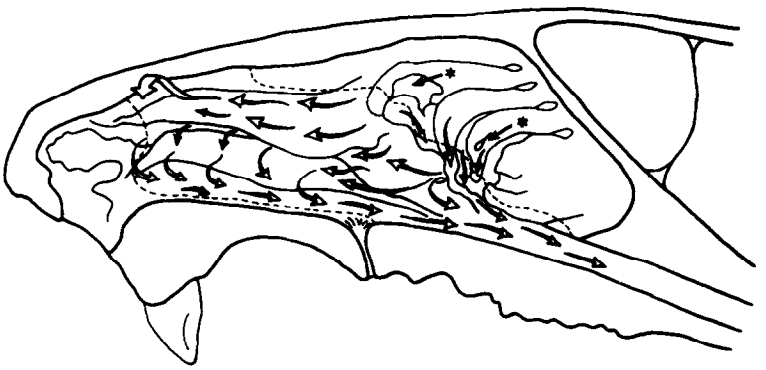

FIG. 3. Diagram showing the direction of mucus flow in the old (2 year) rat nasal cavity. Solid arrows indicated by the asterisks point to two small recessed areas of respiratory epithelium in a region of the ethmoid turbinates which is otherwise covered by olfactory epithelium. These areas are larger in old rats than in young rats.

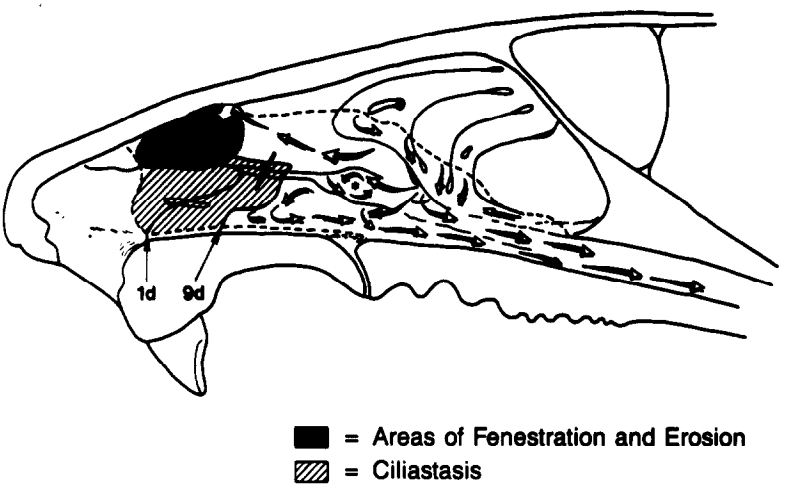

FIG. 4. Diagram of young (10 week) rat nasal cavity following exposure to 175 ppm DMA for 1 or 9 days. The arrows indicate the direction of mucus flow. Note the rotational flow over the posterior lateral wall (asterisk). The solid arrow indicates a region in which mucus flowed from the naso- to the maxilloturbinate over areas of ciliastasis following 9 days of exposure.

some animals exposed for 9 days, mucus was seen to flow across adhesions of the nasoturbinate to the maxilloturbinate, thus bypassing obstruction to normal flow direction presented by fenestration of the ventral margin of the nasoturbinate (Fig. 4). Mean mucus flow rates in the nasopharynx and on the posterior naso- and maxilloturbinates were increased slightly in treated animals compared to controls (Table 1, areas 9, 10, and 5), but this increase was not statistically significant. In the anterior nasal passages, mean mucus flow rates in DMA-treated rats were decreased compared to controls in several areas. However, the only region of the nose in which mean mucus flow rate was significantly decreased in treated rats was in area 1, a site closely adjacent to DMA-induced fenestrations on the anterior nasoturbinates.

DMA-exposed animals: chronic study. Animals exposed chronically to DMA exhibited the same anatomical and functional changes described for chronic control animals (see above). Treatment-induced destruction of nasal tissues included loss of the anterior third of the nasoturbinate and the anterodorsal margins of the maxilloturbinate. Modified mucus flow patterns were found in association with these areas of tissue destruction, as tissue loss prevented normal flow in these areas, with mucus bypassing affected regions (Fig. 5). Other alterations of mucus flow patterns included reversal of flow on the lateral wall in the region posterior to the dorsal margin of the maxilloturbinate (Fig. 5), and in the affected areas the direction of ciliary beat was also modified to the direction of mucus flow. In one animal, the naso- and maxilloturbinates were fused and a thick mucus stream was seen to flow from one turbinate to the other across the point of fusion. Mucus flow rates were generally increased over those of chronic control animals (Table 1). In four out of six animals in the chronic study mucociliary activity resembling that in areas lined by respiratory epithelium was observed as a posterior extension of ciliated epithelium into regions normally lined by olfactory epithelium. This was seen during the visual examination (Fig. 5) and was presumed to represent respiratory metaplasia (see histopathology below).

Histopathology

Controls. Control animals in the acute studies exhibited no abnormalities while rats

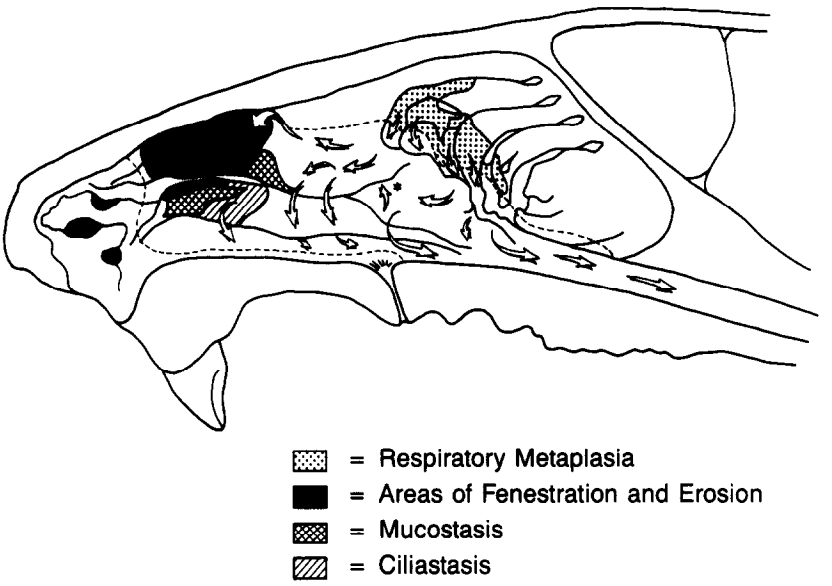

FIG. 5. Diagram of the old rat nasal cavity following 2 years of exposure to 175 ppm DMA. Note that the areas of ciliastasis are similar to those seen following acute exposure (Fig. 4.). Mucus flow patterns were also similar. Altered direction of mucus flow is evident on the lateral wall (*). Note the areas of respiratory metaplasia seen on the ethmoid turbinates. Areas of fenestration and erosion are only slightly larger than those seen following acute treatment.

in the chronic study had varying degrees of chronic inflammation in the nose. The latter lesions were confined to the areas of respiratory mucosa adjacent to the vestibule and comprised accumulations of subepithelial lymphocytes. This chronic inflammatory response was not associated with disruption of the mucociliary apparatus in affected areas.

DMA-exposed animals: acute study. Regional loss of mucociliary function generally correlated well with abnormalities detected in histologic sections. Treatment-induced loss of ciliary activity observed during functional studies was associated with histologic changes ranging from epithelial vacuolation to severe epithelial ulceration and acute or chronic inflammation. Mucostasis was not always associated with histologic evidence of epithelial damage.

Histologic changes associated with acute treatment were found in the squamous, respiratory, and olfactory epithelia. These lesions involved specific regions of the nose but were most severe in the anterior nasal passages. There were characteristic changes in each of the three main epithelial regions.

Squamous epithelium. In the squamous epithelial-lined nasal vestibule there were focal ulcerations on the margins of the anterior extension of the turbinates and adjacent lateral wall (Fig. 6). The breached epithelium was covered by a fibrinocellular coagulum and affected regions exhibited accumulations of neutrophils in the underlying connective tissue. In some animals, the anterior extension of the maxilloturbinate was completely necrotic. Blood vessels in and adjacent to these areas were plugged with thrombi. The squamous epithelium lining the floor of the ventral meati was unaffected.

Respiratory epithelium. Following one or two exposures to 175 ppm DMA there were small epithelial erosions on the dorsal margin of the maxilloturbinate on the lateral scroll and lateral ridge of the nasoturbinate, and on the lateral wall adjacent to these areas. There was also widespread vacuolation of cuboidal and columnar, ciliated and nonciliated respi-

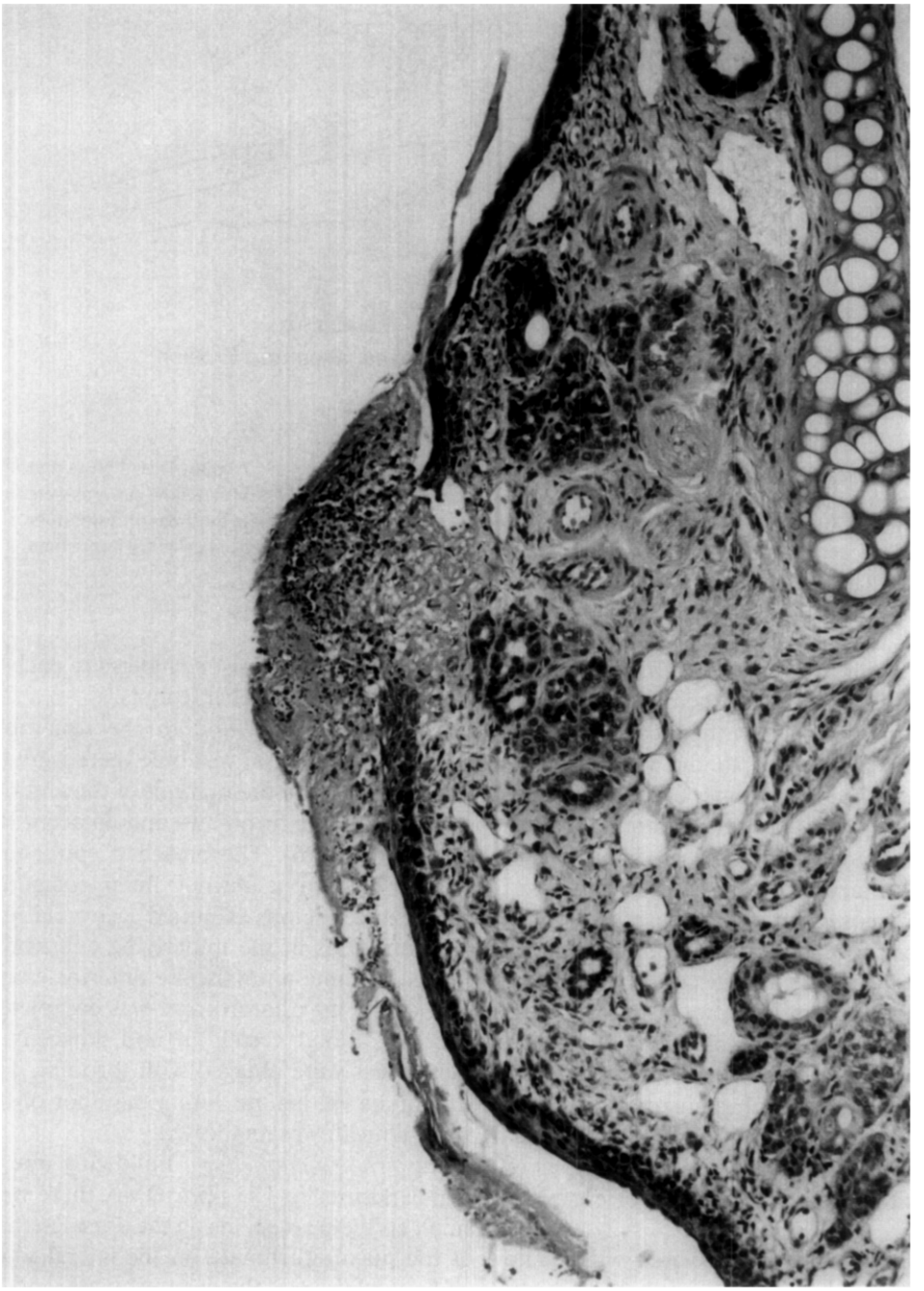

FIG. 6. Light micrograph of squamous epithelium of the anterior lateral wall in a young rat (10 week) exposed to 175 ppm DMA for 4 days. The epithelium is focally ulcerated, with a serocellular plug covering the breached region. (H&E ×350).

ratory epithelial cells. The vacuolated cells were distended and distorted by numerous large and small clear vacuoles, which also involved the underlying glands in severely affected areas (Fig. 7). In areas with epithelial vacuolation there were few remaining cilia, and ciliary activity was absent in functional studies on the same animals. Inflammatory changes, including intravascular margination and focal extravascular aggregates of neutrophils, with serous and cellular exudate in the nasal lumen, were associated with the epithelial erosions but not the vacuolation. These changes were almost entirely confined to the anterior third of the nasal passages. After 4 or 9 days exposure, the vacuolation was less severe or absent, while epithelial erosions and ulceration, combined with inflammation, became progressively more severe and extensive. The almost pure population of neutrophils was later replaced by focal accumulations of neutrophils, monocytes, and macrophages.

Olfactory epithelium. In animals exposed to DMA for 1 or 2 days there was severe vacuolation of olfactory epithelium in the anterior extension of this epithelium into the dorsal meatus. The vacuolation involved both globose and mature olfactory sensory cells, while sustentacular cells were apparently less affected (Figs. 8a and 8b). Following 4 or 9 days exposure there was fairly extensive loss of olfactory sensory cells, but this lesion was again confined to the dorsal meatus. Bowman's glands were unaffected, but in areas of severe sensory cell damage there was some loss of olfactory nerve bundles.

DMA-exposed animals: chronic study. Lesions in rats exposed to DMA for 2 years were very similar to those reported previously for rats exposed for 6 months (Buckley *et al.*, 1985). Only those changes which involve the mucociliary apparatus will be described. Again there was good correlation between the results of functional studies and histopathology. Lesions were most severe in the anterior regions of the nose and comprised focal or regional squamous metaplasia, with the normal respiratory epithelium (Fig. 9a) being replaced by a stratified squamous epithelium (Fig. 9b). This response was associated with chronic active inflammation and occurred in regions with complete loss of mucociliary function. More posteriorly, the only consistent response to exposure was moderate to severe goblet cell hyperplasia, which was most prominent on the posterior lateral wall, a region which exhibited reversal of mucus flow. Respiratory metaplasia seen in four out of six animals in the mucociliary function study was characterized by posterior extension of ciliated respiratory epithelium into regions normally lined by olfactory epithelium. Histologically, these regions resembled normal respiratory epithelium except for the absence of goblet cells.

DISCUSSION

The main aim of this study was to characterize acute and chronic effects of DMA on the rat nasal mucociliary apparatus. However, during the course of the study some of the changes seen in the noses of 2-year-old control and chronically treated rats were considered to be age related. These changes included anatomical differences between young and old rats and darkening of olfactory pigments in older animals. These observations will be discussed first, to aid in interpretation of the DMA-induced effects. Age-related darkening of the pigmentation of the olfactory mucosa has been reported previously in Sprague–Dawley rats (Hinds and McNelley, 1981). Considering the variability of coloration of olfactory epithelium both within and between species, Jackson (1960) concluded that age-related alteration of olfactory epithelial pigmentation is of no functional significance in rats and may be attributable to auto-oxidation of phospholipids in the olfactory epithelium. However, in humans there is a gradual loss of olfactory sensitivity with increasing age (Corso, 1971). Despite the evidence from a wide variety of approaches

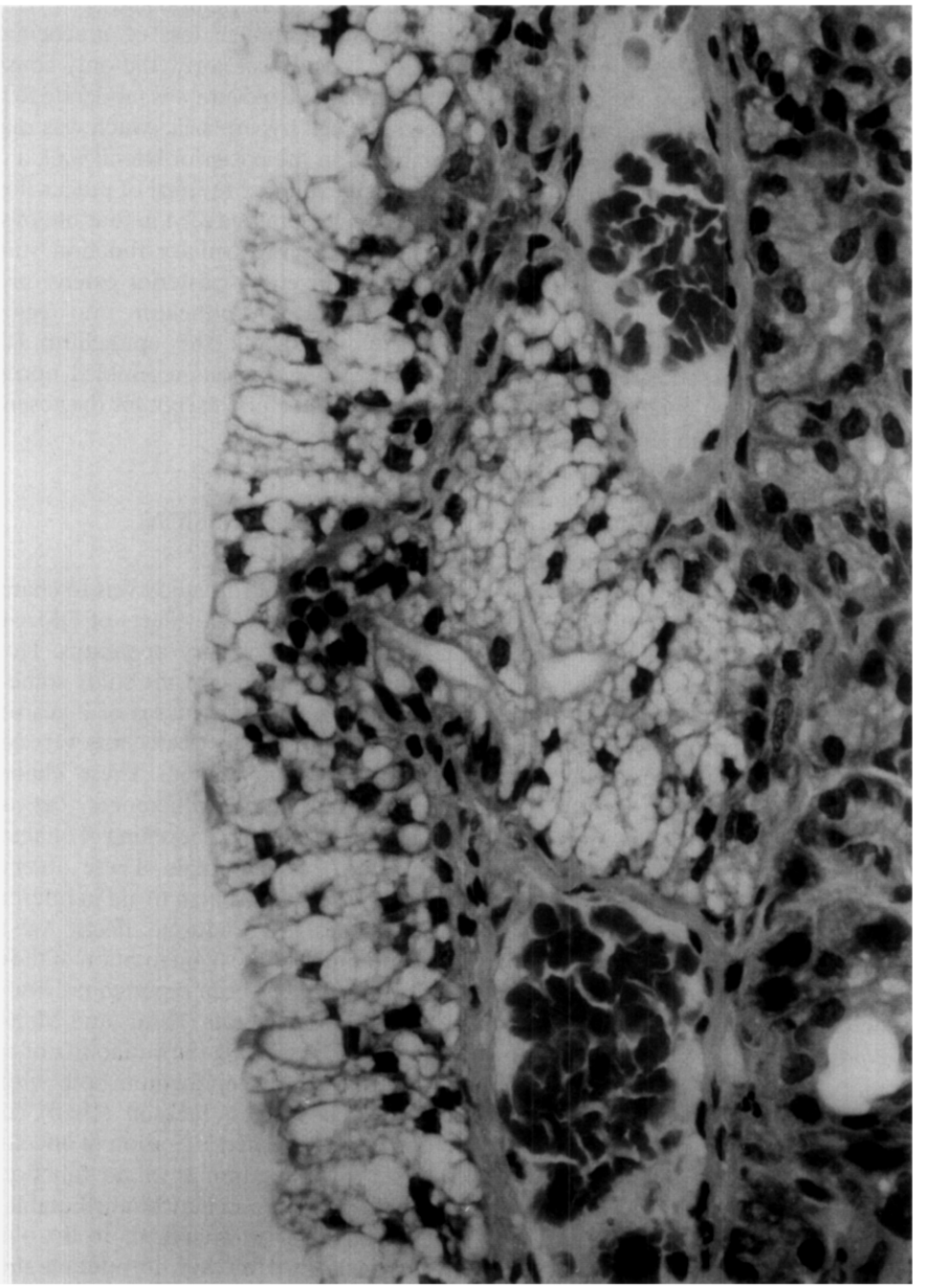

FIG. 7. Light micrograph of respiratory epithelium on the nasoturbinate of a rat exposed to 175 ppm DMA for 2 days. Note extensive vacuolation of both the epithelial cell cytoplasm and the ductular epithelium of the underlying glands. (H&E ×700).

found in the literature, it appears that little conclusive evidence is available on most of the constituents of the olfactory pigment complex (Moulton, 1971). The present study does not address the functional significance of olfactory pigment, but rather provides another observation point for others who may be researching this complex system.

We could find no previous report of age-related anatomical changes in the rodent nasal passages. In the present study there were clear differences between young rats used for acute studies and old rats available from the chronic bioassay, despite the similar genetic backgrounds of the two groups of animals. It was hypothesized that these changes could be due to housing conditions because the chronic study animals were maintained in stainless steel cage racks and were deprived of food each day for the 6 hr of chamber exposure (0 ppm). Recent observations by our group on the nasal anatomy of old F-344 rats that have been housed on bedding and allowed food and water *ad libitum* revealed anatomical features similar to those reported in the aging rats in the present study. This observation indicates that anatomical changes in the nose of the rat are probably not attributable to chamber housing or 6 hr per day of food deprivation. Previous morphometric studies in our laboratory with young adult (7 weeks) and adult (16 weeks) rats demonstrated marked differences in the surface area of the nasal mucosa between the two groups, while there were no significant differences in surface area to volume ratio (Gross *et al.*, 1982). Morphometric information on 2-year-old rats, however, is also not available and was not an aim of this study. The complex structure of the nasal passages, by increasing nasal surface area, plays an important role in nasal functions of humidification, warming, and cleaning of inspired air (Negus, 1958). Furthermore, airflow patterns in the nose probably play a major role in determination of regional exposure levels to inhaled noxious materials, including particulate matter (Torjussen, 1983) and irritant gases (Buckley *et al.*, 1984). The anatomy of the airway has been shown to significantly influence airflow velocity, local turbulance, and residence times for air in casts of the human and baboon nasal cavities (Girardin *et al.*, 1983; Patra *et al.*, 1986). Further work by Morris and Cavanagh (1986) and Aharonson *et al.* (1974) also demonstrated that inspiratory flow rates can affect nasal deposition of vapors in rats and dogs. Changes in these parameters as a result of the aging process could cause age to have an effect on regional dosimetry in the nose and should be kept in mind when studying mechanisms of nasal responses to inhaled materials.

The principal toxic responses to acute DMA exposure in the present study were confined to the more anterior portions of the nasal cavity. Water soluble gases and vapors such as hydrogen fluoride, ammonia, iodine, and formaldehyde are effectively trapped and retained by the moist regions of the upper respiratory tract (Pattle, 1961; Dalhamn and Sjoholm, 1963; Egle, 1972; Morris and Smith, 1982). However, absorption of gases in the upper respiratory tract is influenced by many factors including water solubility, reactivity, and saturation of metabolic pathways and is discussed extensively by Stott *et al.* (1986) and Morris *et al.* (1986). The distribution of lesions found in this study indicates that there is possibly a high rate of deposition of DMA in the anterior nasal passage. McNulty and Heck (1983) showed that following acute exposure, DMA is readily absorbed and retained in highest concentration in the respiratory mucosa, the mucosal type which lines much of the anterior nasal airways. McNulty and Heck (1983) concluded that DMA toxicity is primarily attributable to the irritant properties of the parent compound with a possible role for the metabolites of DMA such as formaldehyde (McNulty and Heck, 1983; McNulty *et al.*, 1983). The direct cytolethal effects of DMA causing frank tissue damage are most likely due to the one or both of these mechanisms; however the mechanisms responsible for the many changes seen

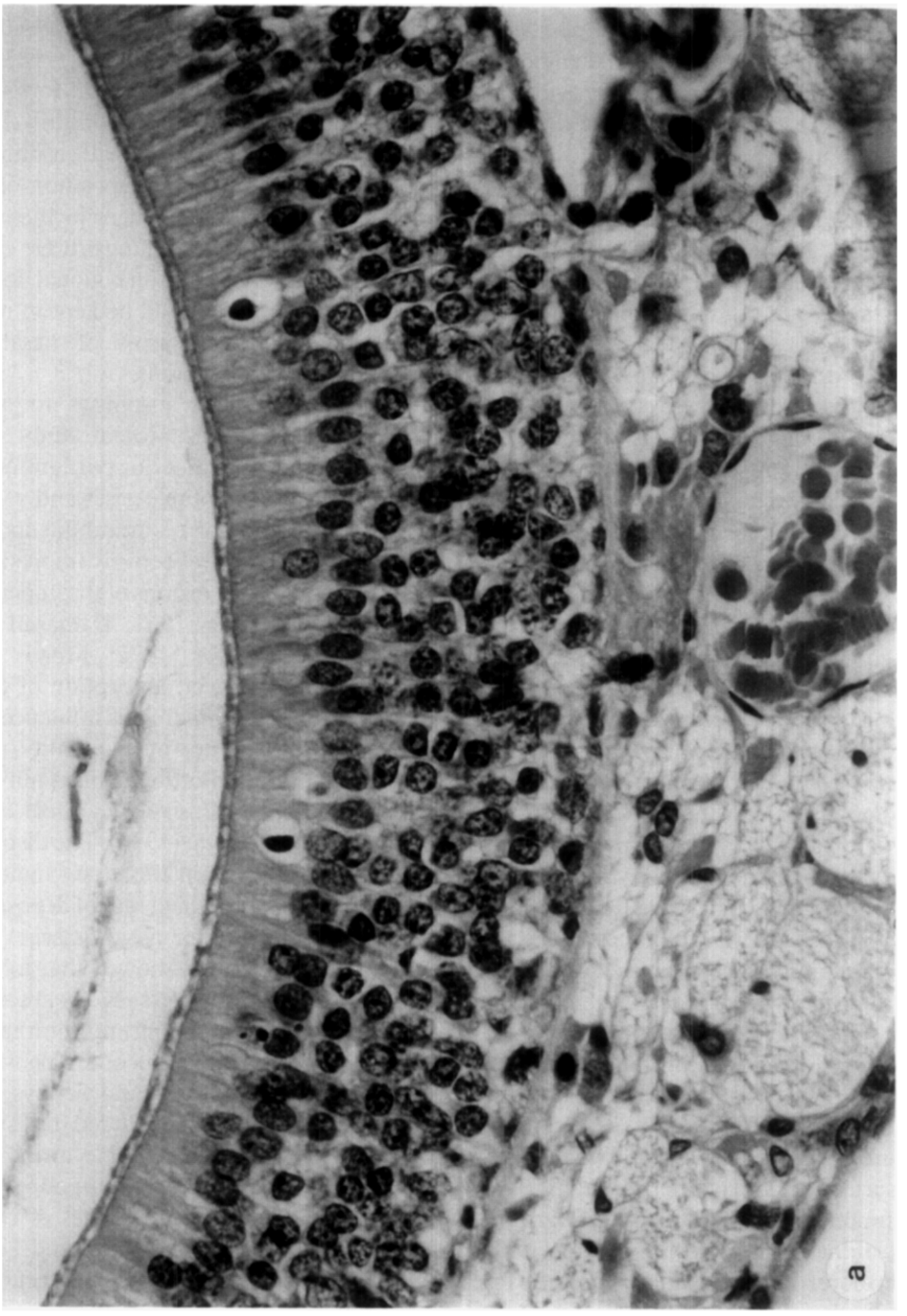

FIG. 8a. Light micrograph of olfactory epithelium in the dorsal meatus of an untreated control young (10 week) rat to show normal structures. (H&E ×700).

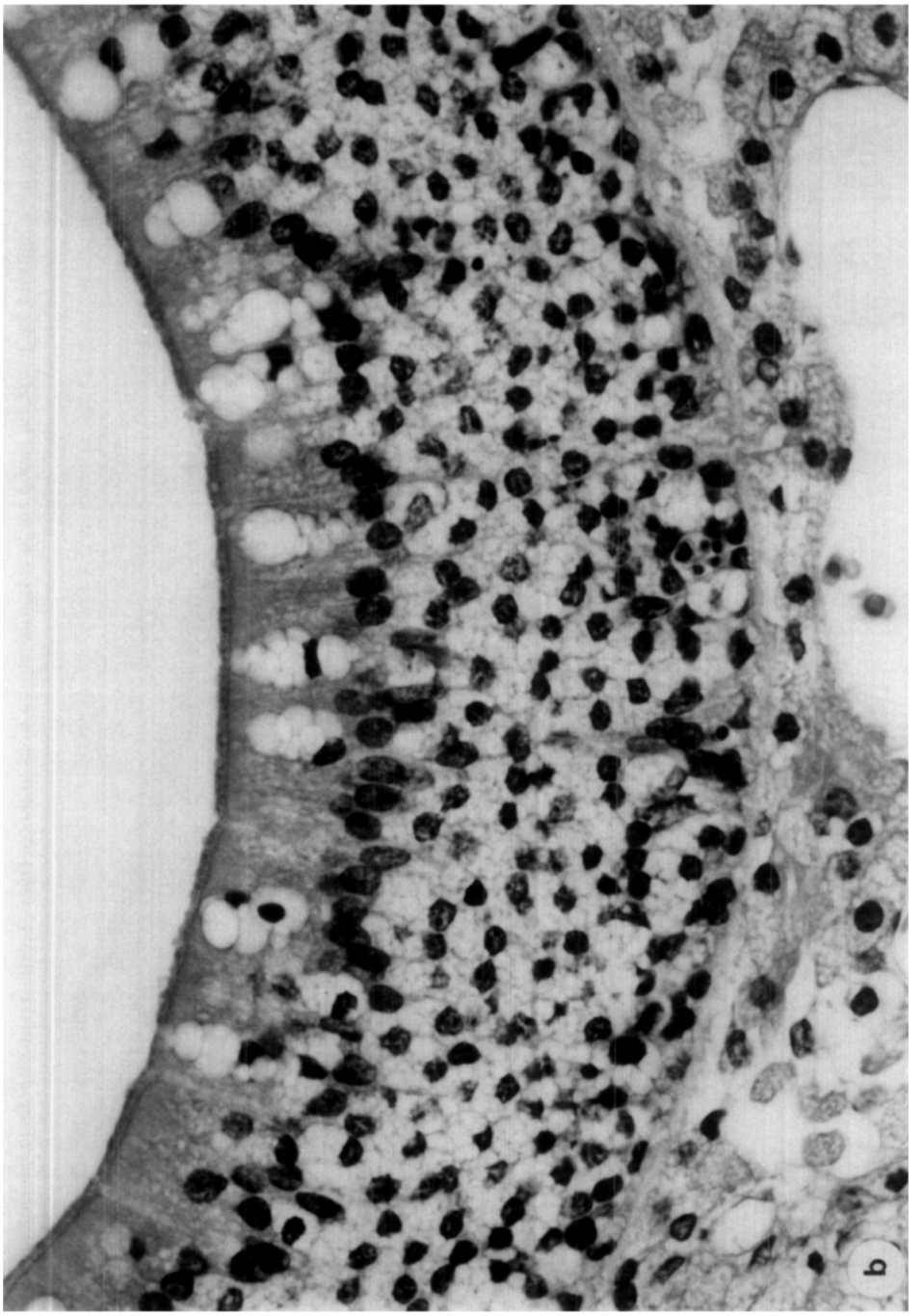

FIG. 8b. Light micrograph of olfactory epithelium of a young (10 week) rat following exposure to 175 ppm DMA for 4 days to show extensive vacuolation of olfactory sensory cells. (H&E ×700).

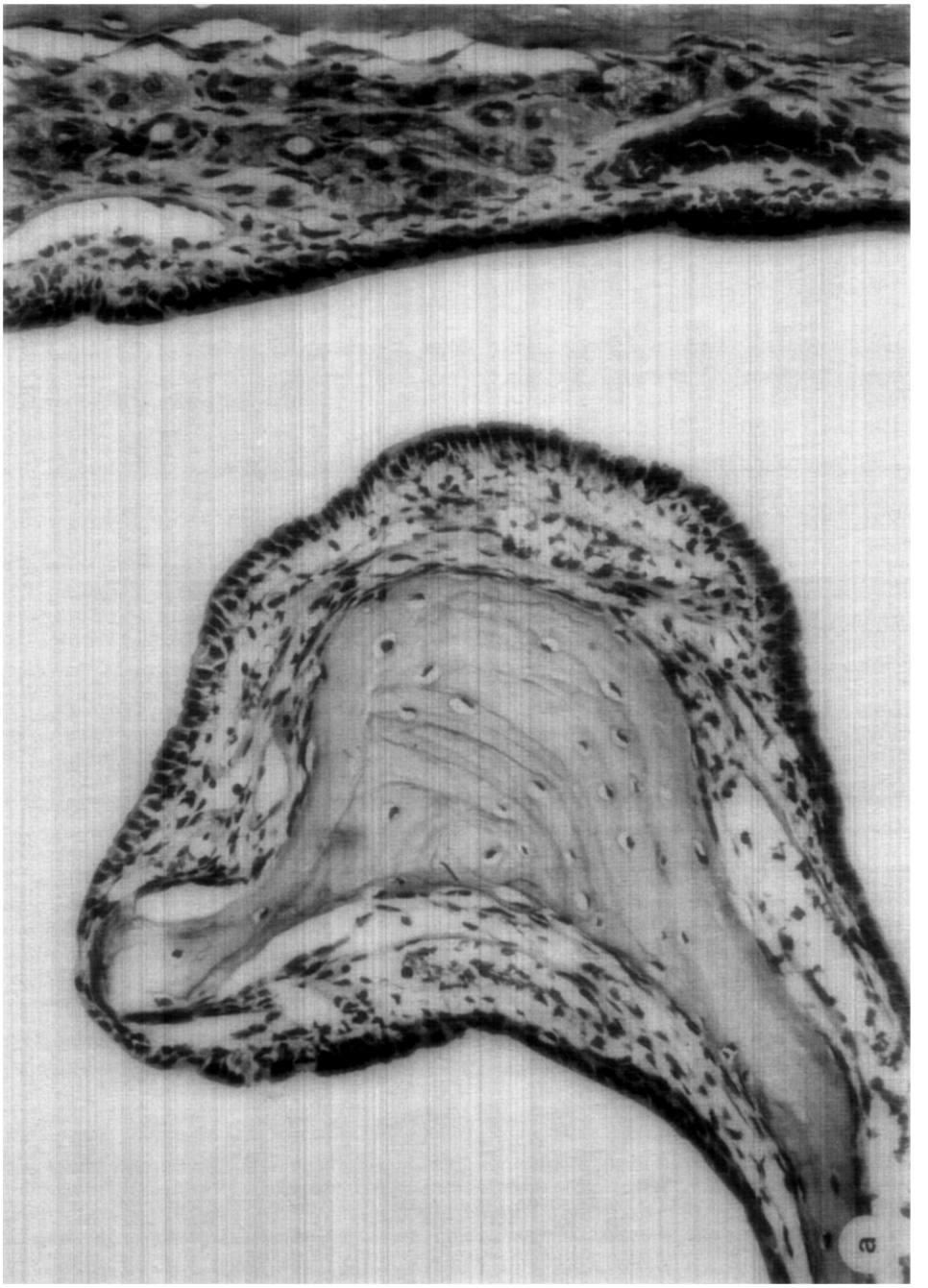

FIG. 9a. Light micrograph of an untreated control (2 year) old rat to show normal structure of the dorsal margin of the maxilloturbinate and adjacent lateral wall for comparison with Fig. 9b. (H&E ×70).

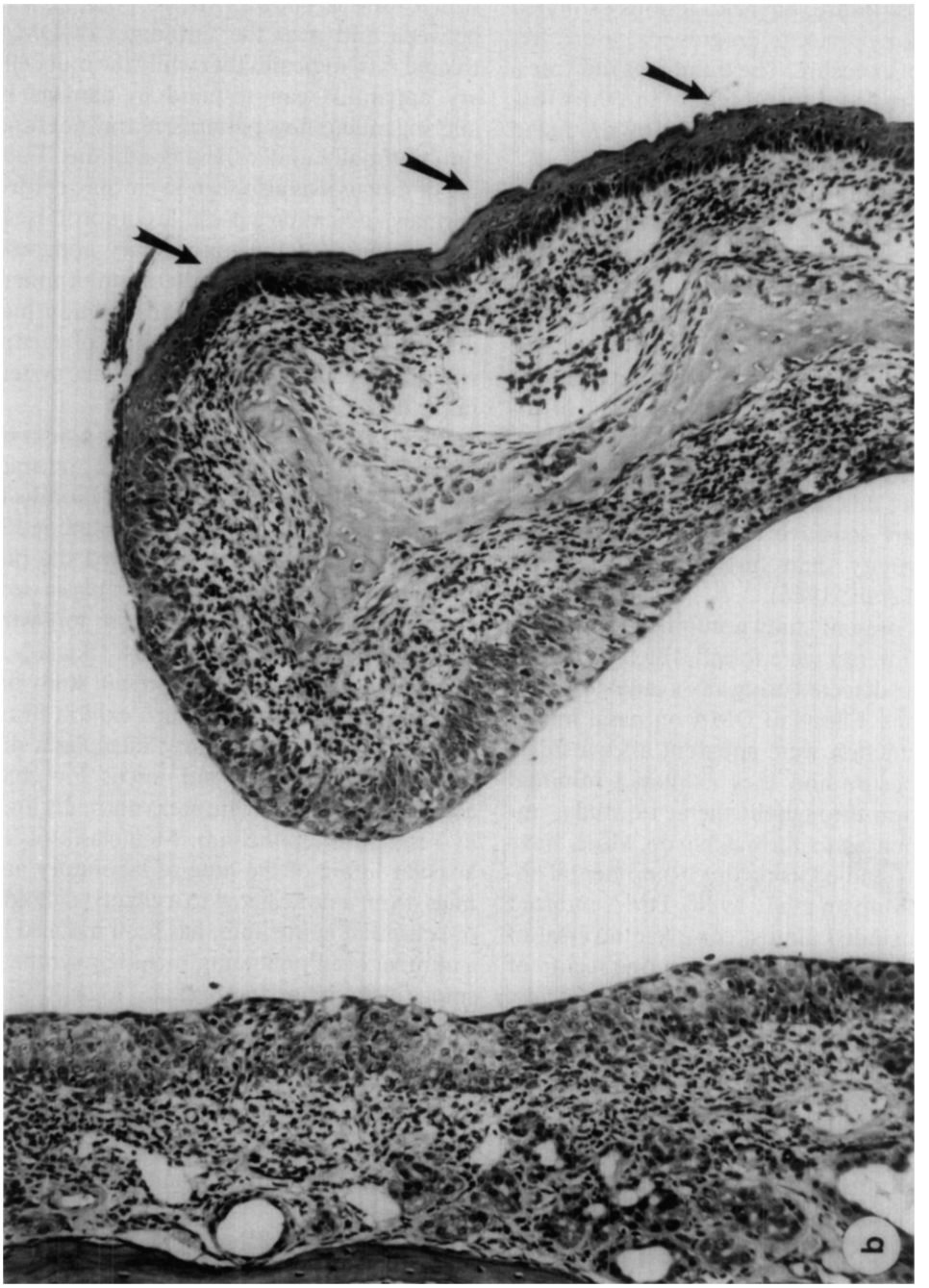

FIG. 9b. Light micrograph of a rat exposed to 175 ppm DMA for 2 years to show thickening and extensive squamous metaplasia of the respiratory epithelium (arrows) and an associated chronic inflammatory response in the underlying lamina propria. (H&E ×70).

in the present study remain to be determined. It is of interest that despite severe tissue destruction in the anterior nose following a single 6-hr exposure, the nasal lesions exhibited very little evidence of progression, even after 2 years of exposure. These findings indicate a possible regional susceptibility to DMA toxicity or a degree of adaptation by the rat to continued DMA exposure.

Postmortem examination of mucociliary function has been used to a very limited extent in the nose. This approach has a number of evident disadvantages because of its invasive nature (Morgan *et al.*, 1984). However, a similar technique was employed to advantage for the assessment of responses to cigarette smoke in the lower respiratory tract of rodents (Iravani and Melville, 1974). Furthermore, visual assessment of mucociliary function using this procedure has been found to be a more sensitive indicator of formaldehyde toxicity than histopathology in rats (Morgan *et al.*, 1986).

In the present study a number of observations of interest were found which could not have been detected using any available *in vivo* techniques. Effects of DMA on nasal mucociliary function were apparent after a single 6-hr exposure and they exhibited minimal progression throughout the acute study, unlike responses to formaldehyde which have also been studied using this experimental approach (Morgan *et al.*, 1986). DMA-induced effects included almost complete reversal of mucus flow and ciliary beat in one region of the lateral wall and clear modification of flow patterns to provide clearance around areas of turbinate and septal destruction. These types of effects have been seen in the tracheobronchial tree of rats following exposure to cigarette smoke (Iravani, 1972) and in cases of chronic bronchitis (Iravani and van As, 1972). In both of the latter studies clearance from the tracheobronchial tree was considered to be impaired. However, we could find no literature on these types of effects in the nasal passages of any species. Altered flow patterns that direct mucus away from areas of ciliastasis raise questions about the mechanisms that control the direction of ciliary beating and mucus flow in the nose, about which little is known. Mucus pooling, seen between and over the turbinates of DMA-treated rats, indicates that while the mucociliary apparatus can respond to damage by altering mucus flow patterns, it may not function optimally under these conditions. Pooling of mucus was also seen in chronic control animals, indicating a possible compromise in the efficiency of the mucociliary apparatus with age. DMA-induced alteration of mucus flow patterns and ciliary beat direction may provide a useful tool for studies of factors which control this complex clearance system in the nose.

Buckley *et al.* (1985) described responses induced in the nasal passages of rats and mice by inhalation exposure for 6 or 12 months to several concentrations of DMA, including 175 ppm. The present study confirmed the previously reported respiratory metaplasia seen in the olfactory region of the nose following chronic exposure of rats to DMA (Buckley *et al.*, 1985). Furthermore, the present study has demonstrated that areas which exhibit histologic evidence of respiratory metaplasia display the ciliary activity and mucus flow characteristics associated with areas normally lined by respiratory epithelium. Metaplasia of the alveolar lining of the lung of laboratory animals, from a nonciliated to a ciliated cuboidal or columnar epithelium, has been induced by a number of air pollutants, including synthetic smog (Nettesheim and Szakal, 1972). It is interesting that metaplasia to a respiratory-type epithelium occurs at both extremities of the respiratory tract. However, it remains to be determined whether these responses are protective in nature or whether similar mechanisms are responsible for these changes in the nose and lung.

A number of findings were reported here for acute DMA exposures, which were not apparent in animals exposed for 6 or 12 months (Buckley *et al.*, 1985), including focal degeneration of the squamous epithelium in

the nasal vestibule and extensive vacuolation of both the respiratory and olfactory epithelia in the anterior nasal passages. Presumably, despite continued exposure, there was considerable repair in the regions lined by squamous epithelium and resolution of the vacuolation seen in the respiratory and olfactory epithelia, following chronic exposure. In the respiratory epithelial-lined regions of the anterior nasal cavity, acute epithelial degeneration led to squamous metaplasia in chronically exposed animals in both the present study and the study reported by Buckley *et al.* (1985), presumably as a protective or adaptive response.

The severe vacuolation of epithelial cytoplasm, induced by acute DMA exposure in the present study, may represent a useful feature for further studies of the mechanisms of DMA toxicity. This change was very severe in affected areas and was seen in both ciliated and nonciliated epithelial cells and ducts of underlying glands of the respiratory mucosa, while in the olfactory epithelium this change was largely confined to sensory cells. Buckley *et al.* (1985) reported vacuolation of olfactory epithelium, which has subsequently been interpreted to involve the ducts of Bowman's glands (K. T. Morgan, unpublished observations). This vacuolation differs significantly from that induced by acute DMA exposure. Epithelial vacuolation is a common artifact in many tissues (Thompson and Luna, 1978). However, epithelial vacuolation was not seen in control animals in the present study, and similar changes have not been seen in many other studies of nasal toxicity carried out in this laboratory using similar or identical histologic procedures, suggesting that it was a response to DMA exposure. It has been proposed that toxic responses to DMA may be attributable to the metabolism of this compound to formaldehyde (McNulty *et al.*, 1983). However, acute formaldehyde toxicity in rats was not found to induce epithelial vacuolation or the severe subepithelial destructive changes seen with DMA in the present study (Morgan *et al.*, 1986), suggesting that an alternative mechanism may be responsible for DMA toxicity. Further studies of DMA-induced epithelial vacuolation may provide further insight into the mechanism of action of this chemical in the nose of the rat.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the CIIT inhalation staff for assistance with DMA exposures and Susan Sadler for her excellent artwork.

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