MICROSCOPY RESEARCH AND TECHNIQUE 24:195–213 (1993)

Morphological Study of the Effects of Intranasal Zinc Sulfate Irrigation on the Mouse Olfactory Epithelium and Olfactory Bulb

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KEY WORDS Deafferentation, Regeneration, Sensory afferents, Electron microscopy, Olfactory receptor cells

ABSTRACT The effects of intranasal zinc sulfate ($ZnSO_4$) irrigation on the morphology of the olfactory epithelium and olfactory bulb were studied in mice with short survival times (as early as 1 day) and with long survival times (up to 593 days) after the irrigation procedure. As in several previous studies, the olfactory epithelium was completely destroyed within a few days after the $ZnSO_4$ treatment. Within 2–4 days, the septum and turbinates were covered by a new, cuboidal epithelium, the cells of which differed significantly from any cells normally seen in the olfactory epithelium. Slowly, over several months, small areas of the olfactory epithelium regenerated in many of the animals.

The ultrastructural changes occurring in the olfactory bulb from 1 to 25 days (the reactive stage) were characterized by degenerating olfactory axons and axon terminals, hypertrophy of astroglial cell processes, and proliferation of or extravasation by phagocytic cells. By 25 days after intranasal $ZnSO_4$ irrigation, the number of reactive glial processes and phagocytic cells returned to normal. In some mice with survival times of 150 days or longer, there was reinnervation of small areas of the olfactory bulb by regenerated olfactory axons. These new olfactory axons innervated only superficial glomeruli or the outer portions of deeper glomeruli, but they formed synaptic contacts with mitral/tufted cells and periglomerular cells that did not differ from control animals. These findings were supported by tract-tracing experiments with ^3H-amino acids and by behavioral analysis.

In summary, the ultrastructural changes observed in the olfactory bulb in this study were not significantly different from those observed after surgical lesions of the olfactory epithelium or nerve. The olfactory bulb, however, never fully recovered; glomeruli remained shrunken (though with normal dendro-dendritic synaptic connections), and there was minimal olfactory axon reinnervation.

INTRODUCTION

Several studies have found that a variety of substances administered to the nasal cavity are taken up by the olfactory receptor cells and transported to the olfactory bulb. These substances include infective agents such as poliomyelitis virus (Howe and Bodian, 1940), neuronal tracers such as horseradish peroxidase and Evans Blue-labelled albumin (Kristensson and Olsson, 1971), and metals such as cadmium (Gottofrey and Tjälve, 1991) and colloidal gold (DeLorenzo, 1970). Tritiated amino acids can be transported as peptides (Burd et al., 1982) or presumably as larger molecules (Burd, 1979; Land and Shepherd, 1974; Weiss and Holland, 1967). Given this uptake and transport process, it is possible that a toxic chemical applied to the nasal cavity might produce degenerative changes in the olfactory nerve terminals which differ from the changes observed following the surgical destruction of the olfactory epithelium (Estable-Puig and DeEstable, 1969; Pinching and Powell, 1972). In addition, since the extracellular space of the olfactory epithelium and the subarachnoid space of the olfactory bulb may be continuous (Jackson et al., 1979), it might be possible for a toxic substance to cause direct damage to the olfactory bulb.

Intranasal zinc sulfate ($ZnSO_4$) irrigation has been used by several investigators as a method to destroy the olfactory receptors and thus eliminate olfactory sensitivity (Alberts, 1974; Murphy, 1976). In addition, in the late 1930s it was clear that poliomyelitis virus could enter the monkey central nervous system (CNS) from the nasal mucosa (Schultz and Gebhardt, 1937). Protection from polio was achieved by pretreating monkeys with intranasal $ZnSO_4$ (Schultz and Gebhardt, 1937, 1942). This protection was directly related to the ability of $ZnSO_4$ to destroy the olfactory epithelium. In fact, during polio outbreaks in Toronto (1937) and Nic-

Received March 10, 1991; accepted in revised form August 23, 1991.

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aragua (1938), when polio vaccines were not yet available, several thousand patients (mostly children) were treated with $ZnSO_4$ as a prophylaxis for poliomyelitis (Tisdall et al., 1938; also see discussion in Schultz and Gebhardt, 1942). The prophylactic value of these treatments was questionable since most patients did not become anosmic (Schultz and Gebhardt, 1938; Tisdall et al., 1938).

Adverse effects of intranasal $ZnSO_4$ administration on the physiology (Troitskaya and Gladysheva, 1990) and morphology of the olfactory epithelium (Cancalon, 1982; Harding et al., 1978; Matulionis, 1975, 1976; Mulvaney and Heist, 1971; Schultz, 1960; Smith, 1938, 1951) and olfactory bulb (Harding et al., 1978; Margolis et al., 1974), and on the biochemical (Baker et al., *1983*; Biffo et al., 1990; Harding et al., 1978; Margolis et al., 1974; Nadi et al., 1981) and molecular (Ehrlich et al., 1990) changes in the olfactory bulb have been examined. Ultrastructural studies have been conducted on the effects of intranasal $ZnSO_4$ administration on the olfactory epithelium (Cancalon, 1982; Matulionis, 1975, 1976; Mulvaney and Heist, 1971). The effects of this procedure on the ultrastructure of the olfactory bulb, however, have only appeared in a dissertation (Burd, 1979). It is not known whether intranasal administration of $ZnSO_4$ has any direct effects on neurons in the olfactory bulb.

The purpose of the present study was to examine the acute and chronic changes in the olfactory epithelium and the olfactory bulb of the mouse following chemical destruction of the olfactory epithelium by intranasal $ZnSO_4$ irrigation in order to: 1) assess the degree of degeneration and regeneration in the olfactory epithelium and in olfactory axon projections to the olfactory bulb and 2) determine whether this deafferentation procedure produces degenerative changes in the olfactory bulb that are comparable to those of surgical lesions to the olfactory epithelium and olfactory nerve. I demonstrate here that the $ZnSO_4$ irrigation procedure developed by Margolis and colleagues (Harding et al., 1978; Margolis et al., 1974; Nadi et al., 1981) is a reliable method for producing complete peripheral deafferentation of the olfactory bulb and that the effects of intranasal $ZnSO_4$ irrigation on the olfactory bulb are essentially the same as those produced by surgical destruction of the olfactory epithelium and olfactory nerve transection.

MATERIALS AND METHODS

$ZnSO_4$ Irrigation Procedure

Female white mice of a random bred strain (Flow Laboratories), CD-1 strain, or Swiss Webster strain (Charles River Breeding Lab) were used in this study. Experimental mice received a 0.1 ml intranasal irrigation of either physiological saline (0.9%) or 0.17 M $ZnSO_4$. A 1 cc tuberculin syringe with a no. 27 needle, shortened to 3 mm and filed smooth, was used to administer the solutions to the nasal cavity. The procedure (Harding et al., 1978; Margolis et al., 1974) consisted of inserting the needle through the right external nares and rapidly expelling the contents of the syringe into the nasal cavity.

Behavioral Testing Procedure

Mice raised in the animal facility at the University of North Carolina did not develop rhinitis infections, and mice purchased from outside suppliers were tested to eliminate mice with rhinitis infections from this study. Simple behavioral tests were performed on untreated mice and mice before intranasal administration, 3 and 10 days after the treatment, and just before perfusion to assess basic olfactory sensitivity. The test consisted of fasting mice for 17–24 hours and testing their ability to find a food pellet buried under clean bedding within 5 minutes (Harding et al., 1978). A positive score indicated that the mouse probably located the food pellet using chemosensory cues; a negative score indicated that either the mouse could not smell or was not motivated to perform the task. Animals with negative scores prior to treatment were not used in the study.

Light Microscopy on the Nasal Capsules

The nasal capsules from most of the animals whose brains were fixed for electron microscopy (see below) were also prepared for light microscopy. The bony snout was decalcified in a solution of 25% formic acid with 7.5% citrate for 3–4 days, neutralized for a day in 5% sodium sulfate, rinsed overnight in running water, and embedded in paraffin. Serial 10 micron sections were stained with cresyl violet.

Transport of Tritiated Amino Acids and Autoradiography

Tritiated amino acids were applied to the nasal cavities for two reasons. First, experiments were designed to replicate the procedures used to administer saline and $ZnSO_4$, with the underlying assumption that tritiated amino acids in an aqueous solution would be distributed throughout the nasal cavity in the same way that the treatment solutions were distributed. The end result would be visualization of this distribution pattern through the use of autoradiography. Second, these experiments were done to identify, in normal and experimental animals, the presence or absence of olfactory receptor cells with axonal projections to the olfactory bulb. Land and Shepherd (1974) showed that tritiated amino acids placed onto small regions of the olfactory epithelium were taken up by olfactory receptor cells, incorporated into larger molecules, and transported along the olfactory axons to the olfactory bulb. Our experiments were designed to label all regions of the olfactory epithelium that could be exposed from our intranasal irrigation procedure. Fourteen $ZnSO_4$-treated mice, 6 saline-treated mice, and 4 untreated control mice were used in this part of the study. The survival times for the $ZnSO_4$-treated mice were 4 ($n = 2$), 5, 11 ($n = 2$), 49, 121, 151, 174, 232 ($n = 2$), 236, and 593 ($n = 2$) days, and the survival times for the saline-treated mice were 4 ($n = 2$), 11, 49, 232, and 600 days.

All animals received intranasal administration of equal amounts of ^{3}H-proline and ^{3}H-leucine (0.1 ml of 25 or 50 μCi/μl) in exactly the manner used to administer the $ZnSO_4$. Mice in each of the treatment groups were anesthetized after 6–8½ hours and perfused with

TABLE 1. Results of behavioral testing[1]

	Pretreatment		3 or 4 days		10 days		60 days	
Group	+	−	+	−	+	−	+	−
Control	66 (86%)	11 (14%)						
Saline			12 (80%)	3 (20%)	10 (83%)	2 (17%)	8 (100%)	0
$ZnSO_4$			0	16 (100%)	0	12 (100%)	8 (33%)	16 (67%)

[1]Number of and % of total animals able to find a food pellet buried under clean bedding within a 5 minute test given at specific days after the treatment. Some animals were tested on several of the survival times indicated. + represents animals able to find the food pellet; − represents animals unable to find the food pellet.

formalin (3.7% formaldehyde in deionized water). Formalin was selected as a fixative solution because, unlike glutaraldehyde, it does not cross-link free amino acids within the tissue. Thus, only larger, newly synthesized molecules should contain the ^3H label.

After perfusion, nasal capsules and olfactory bulbs were removed and stored in fixative solution for an additional 10–14 days. Following fixation, the nasal capsules were decalcified and embedded in paraffin as described above; the olfactory bulbs were also embedded in paraffin. Both sets of tissues were sectioned (10 μm) with a rotary microtome, and the sections were placed on glass slides and processed for autoradiography. The autoradiography procedure included coating the slides with NTB-2 nuclear tract emulsion (Kodak), exposing the slides in light-tight boxes at 4°C for 2–5 weeks, developing the slides in 17 to 19°C D-19 (Kodak) for 2 minutes, fixing the slides in cold fixer (Kodak), rinsing and counterstaining the slides with 0.1% cresyl violet or 1% thioinine, and coverslipping with DPX mounting medium. Darkfield photomicrographs were used to illustrate the distribution of autoradiographic silver grains, thus indicating the location of ^3H-labelled molecules in the nasal capsules and olfactory bulbs; brightfield microscopy added data for light microscopic analysis of the olfactory epithelium.

Electron Microscopy Methods

The olfactory bulbs from 33 $ZnSO_4$-treated mice (1, 2, 4, 5 [n = 3], 6, 8, 11, 14, 22 [n = 2], 25 [n = 2], 45, 51, 81, 91, 123, 137, 151, 162, 258, 353, 370 [n = 2], 415, 426, 427 [n = 2], 448, 472, and 553 days survival), 6 saline-treated mice (4, 6, 8, 162, 231, and 236 days survival), and 12 control, untreated mice were prepared for light and electron microscopy. Animals were anesthetized by an intraperitoneal injection of a Nembutal solution and perfused with 1% paraformaldehyde and 1% or 3% glutaraldehyde in 0.12 M phosphate buffer with 0.02 mM $CaCl_2$. Blocks of olfactory bulb tissue received a secondary fixation in buffered 2% osmium tetroxide. The tissue was embedded in a 6:4 ratio mixture of Spurr and Epon epoxy resins (Colman et al., 1976). For light microscopic examination, semi-thin (0.5–1 μm) sections were cut and stained with 0.1% toluidine blue in 1% sodium borate. Ultra-thin sections were stained with 5–7% aqueous uranyl acetate followed by lead citrate (Reynolds, 1963, or Sato, 1968) and examined with a JEM 100B or a Siemens 101 electron microscope. Identification of olfactory bulb structures was aided by descriptions from previous reports (Pinching and Powell, 1971b,c,d; Price and Powell, 1970a,b,c). Observations concentrated on the olfactory nerve layer and glomerular layer.

RESULTS

Behavioral Testing

Immediately after intranasal irrigation with saline or $ZnSO_4$, animals displayed sneezing and sniffing behaviors, and $ZnSO_4$-treated mice continued to display this sniffing behavior throughout the study. Zinc sulfate eliminated the olfactory behavior tested here at short survivals (3–10 days), but olfactory behavior returned in some animals by 60 days. Saline treatment also eliminated positive behavioral responses in 17–20% of the animals at short survival times (3–10 days), but all animals could perform the task after 60 days. Morphological examination of the nasal capsule (see below) suggested that there was mechanical disruption of the olfactory epithelium in some of the saline-treated animals. The results of behavioral testing on $ZnSO_4$- and saline-treated mice are shown in Table 1. None of the $ZnSO_4$- or saline-treated mice in this study died as a result of the intranasal irrigation.

Light Microscopy of the Olfactory Epithelium and Lamina Propria

Untreated and Saline-Treated Mice. The normal structure of the mouse olfactory epithelium and lamina propria has been studied previously (Adams, 1972; Frisch, 1967; Harding et al., 1977), and the structure is only briefly described here to provide a comparison for the animals with experimental treatments. The morphology of the olfactory epithelium lining the septum, dorsal fossa, and turbinates was basically the same, differing only in thickness (Fig. 1). The structure of the olfactory epithelium and lamina propria in the adult mouse is shown in Figure 2A and the respiratory epithelium is shown in Figure 2B.

At short survival times (4–11 days), intranasal administration with saline disrupted the surface structure of the olfactory epithelium on the treated (right) side (Fig. 2C), but similar disruption was not apparent on the untreated (left) side or in animals with longer survival times (49–600 days). The disruption was likely due to mechanical damage from the force of fluid moving through the nasal cavity.

$ZnSO_4$-Treated Mice. Unilateral intranasal $ZnSO_4$ administration usually affected right (treated) and left nasal cavities equally. Two days after $ZnSO_4$ irrigation, the olfactory epithelium lost its columnar organization and, in most regions, separated from the lamina propria (Fig. 2D). Changes occurring in the nasal cav-

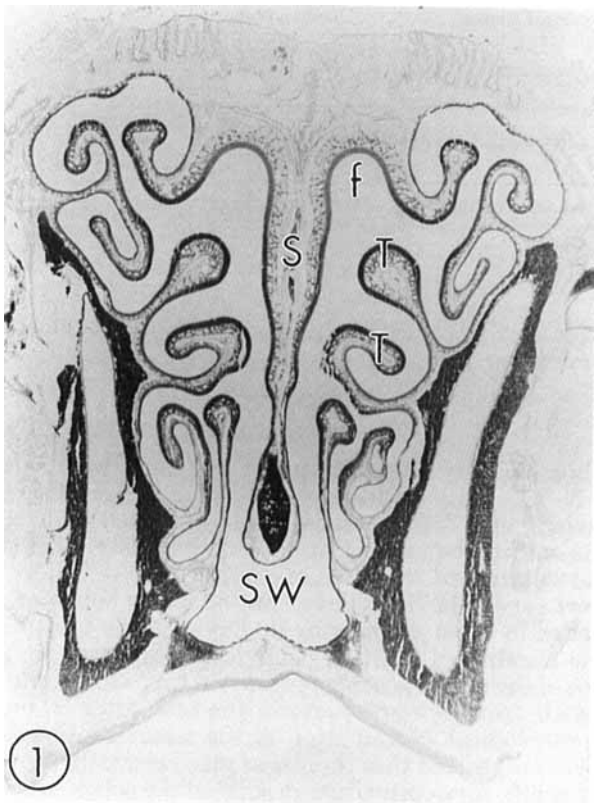

Fig. 1. Coronal section through the nasal cavity at the level of the septal window. Olfactory epithelium lines the dorsal fossa (f), most of the septum (S), and much of the area covering the turbinates (examples at T). Right and left sides of the nasal cavity are connected via the septal window (SW), but the two sides are separate at more rostral and caudal levels. ×14.

ity over time are illustrated in Figure 2D–H. Large, pale, cuboidal cells covered the lamina propria within 2–4 days; these cells were not derived from the normal olfactory epithelium since the cells of the olfactory epithelium remained small and densely stained even as they separated from the lamina propria (Fig. 2E). The large cells and later (11–25 days) the small, densely stained cells appeared to originate from the lamina propria (Fig. 2F,G). These small cells were very similar in morphology to the cells in the normal olfactory epithelium (Fig. 2A) and regenerated olfactory epithelium (Fig. 2H).

In 60% (8 out of 13) of the mice that were examined 151–593 days after intranasal $ZnSO_4$ irrigation, small patches of regenerated olfactory epithelium were observed (Fig. 2H). In no case did the olfactory epithelium completely regenerate throughout the nasal cavity. The regenerated regions were usually located in the left dorsal fossa, but occasionally they were also present on the left or right turbinates. The regenerated epithelium was generally thinner than normal, but had the same basic structure as normal olfactory epithelium.

Autoradiography of the Nasal Capsules and Olfactory Bulb

Untreated and Saline-Treated Mice. In the nasal capsules, silver grains covered both respiratory and olfactory epithelium and the underlying lamina propria (Fig. 3A,F), but rarely labelled sensory epithelium in the vomeronasal organ (Fig. 3F). In the olfactory bulb, silver grains were located only over the olfactory nerve layer and glomerular layer (Fig. 3C), but not over deeper layers of the bulb; little or no label was present in the accessory olfactory bulb. In most control and saline-treated animals, the label was uniformly distributed within the right nasal capsule, but complete labelling over the epithelium and olfactory bulb on the left (untreated) side was less predictable (Fig. 3A,C). Thus, comparison of labelling in the epithelium and the bulb revealed that the left dorsal fossa and septum form significant axonal projections to the medial area of the left olfactory bulb.

$ZnSO_4$-Treated Mice. Fourteen $ZnSO_4$ treated mice were examined in this experiment, but only 2 of these mice had any label in the olfactory bulb. One mouse (151 days survival) had dense labelling in the olfactory nerve and glomerular layer along part of the lateral border of the right olfactory bulb. The olfactory nerve layer and the glomerular layer of this region were considerably thinner than normal. The left olfactory bulb of the other mouse (593 days survival) was labelled along the medial and the dorsolateral borders (Fig. 3D), however, the labelled olfactory nerve and glomerular layers were thinner than in controls and the intensity of labelling was less. In this animal, a corresponding patch of olfactory epithelium was identified in the left nasal capsule (Fig. 3B).

As in the normal and saline treated animals, a dense layer of silver grains covered the turbinates, septum, and dorsal fossa in the right and most of the left nasal capsule. In some mice with short survival times (4–11 days), however, the presence of large clumps of cells filling the spaces between the turbinates (Fig. 3E), and with longer survival times (121–593 days), the structural rearrangement of the nasal capsule, may have prevented the tritium from contacting regions of the nasal capsule that may have contained olfactory epithelium (Fig. 3B).

In summary, the administration of tritiated amino acids to the right nasal cavity and the subsequent, nearly uniform distribution of silver grains on the right side of the nasal capsule and the right olfactory bulb indicate that all areas of the right olfactory bulb were likely to be similarly affected by any intranasal treatment. Much of the left nasal capsule was also labelled as fluids gained access to the left side via the septal window (see Fig. 1). In general, it seemed that the area of the left nasal capsule lesioned by $ZnSO_4$ was more complete than the area of nasal capsule that was heavily labelled with ^3H. Perhaps the mechanics of degenerating olfactory epithelium adjacent to only mildly affected olfactory epithelium are such that the mildly affected areas are removed from the lamina propria when adjacent, more affected areas slough off. To maintain consistency with respect to the effects of in-

tranasal administration of saline or $ZnSO_4$, however, the results that follow pertain only to the right olfactory bulb, unless otherwise specified.

Light Microscopy of the Olfactory Bulb

Untreated and Saline-Treated Mice. The basic structure of the main olfactory bulb (MOB) has been studied previously (Ramón y Cajal, 1955a,b; see review by Shepherd, 1972). The olfactory bulb is composed of six concentric layers: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), and granule cell layer (GRL) (Fig. 4A). The olfactory axons travel along the surface of the bulb before penetrating and terminating in the olfactory glomeruli (Fig. 4C).

In the MOB of the mouse, each glomerulus was a spherically shaped neuropil (60–160 μm in diameter) and contained olfactory receptor cell axons and terminals and dendrites of mitral, tufted, and periglomerular cells. Toluidine blue stained the olfactory axons deep blue while only lightly staining other axons and dendrites. Therefore, groups of olfactory axons within the glomeruli were readily identified. Olfactory axons were not observed in the periglomerular region or in the deeper layers of the MOB.

Saline irrigation of the nasal cavity resulted in minimal changes in the ONL and no observable changes in the deeper layers of the MOB at 4 and 6 days (Fig. 4B); at longer survival times, the morphology was not different from untreated animals.

$ZnSO_4$-Treated Mice. The effects of intranasal $ZnSO_4$ irrigation on the MOB were similar to those reported by Harding and coworkers (1978). $ZnSO_4$ administration resulted in the loss of the ONL and shrinking of the glomeruli. These two features indicate that the olfactory receptor cell axons degenerated. However, the neurons in the GL maintained their periglomerular distribution. The changes that occurred in the MOB after intranasal $ZnSO_4$ administration appeared to be limited to the ONL and glomeruli; other layers did not appear to be affected. Loss of olfactory axons was apparent in both the right and left MOB, but only the right (treated) side was used for the following analysis.

In mice with survival times ranging from 1 to 14 days, large spaces were observed with increasing frequency in the ONL and neuropil of the MOB glomeruli. This disruption was apparent in the ONL as early as 1 day after the lesion, and, by 6 days after the lesion (Fig. 4D), the olfactory axons no longer formed a compact layer at the surface of the olfactory bulb. Also observed in the ONL during this period were cells not present in the unlesioned animals. Cells of one type were variable in size, frequently larger than the dark, oblong glial cells of the ONL, and, as the most distinguishing feature, contained numerous vacuoles and dense granules (Fig. 4E). Another type of cell was characterized by dense round nuclei and dense cytoplasm. This second type of cell as well as cells with flat irregularly shaped nuclei occasionally surrounded blood vessels at the surface of the olfactory bulb and in the ONL and GL (Fig. 5A).

While the ONL was disrupted, olfactory axons were still present in the MOB of some mice up to 14 days survival. In 9 out of 10 mice with survival times ranging from 22 to 137 days after $ZnSO_4$ administration, the ONL and glomeruli of the right MOB appeared to be devoid of olfactory axons (Fig. 5B). However, in most mice (6 out of 9) with survival times of 151 days or greater, bundles of olfactory axons were present in small areas of the ONL and GL of the right MOB (Fig. 5C). These areas appeared to be sites of reinnervation since the axons entered only superficial glomeruli, and these glomeruli were smaller than most glomeruli in control animals. Reinnervated glomeruli were not evenly distributed throughout the right MOB. The areas most frequently reinnervated occurred in the ventrolateral portion of the bulb. Most areas of the MOB, however, were not innervated by olfactory axons even 472 days after the $ZnSO_4$ lesion.

Electron Microscopy

Untreated and Saline-Treated Mice. The normal ultrastructure of the MOB has been studied in several mammals including the cat (Willey, 1973), rat (Andres, 1965; Hinds, 1970; Pinching and Powell, 1971b,c,d; Price and Powell, 1970a,b,c; Reese and Brightman, 1970), and mouse (Burd, 1980; Hirata, 1964; White, 1972). The ONL contained bundles of 10–100 unmyelinated olfactory axons surrounded by thin cytoplasmic processes of ensheathing glial cells scattered throughout this layer. A few microglial cells were observed and identified by nuclei with dense chromatin and perikarya containing long cisternae of rough endoplasmic reticulum and numerous residual bodies. Dense electron-opaque rings within glial cell processes were also present, but were observed more often in the $ZnSO_4$-treated mice (see below).

Bundles of olfactory axons penetrated into the glomeruli and formed many synaptic contacts with mitral, tufted, and periglomeruluar cell dendrites. Terminals of olfactory axons were quite electron dense and were filled with spherical agranular vesicles, contained mitochondria, and formed asymmetric synaptic contacts with periglomerular cell dendrites and with mitral/tufted cell dendrites (Fig. 6A).

In most areas of the ONL and GL of saline-treated mice, the ultrastructure was indistinguishable from untreated mice. Eight days after saline administration, however, several olfactory terminals contained swollen, distorted synaptic vesicles; dense, shrunken terminals were not observed.

$ZnSO_4$-Treated Mice. Chemical destruction of the olfactory epithelium induced a series of ultrastructural alterations on the ONL and GL that could be divided into a "reactive stage" and a "recovery stage." Briefly, the reactive stage, roughly from 1 to 25 days after the lesion, was characterized by degenerating olfactory axons and terminals, hypertrophy of astroglial cell processes, and an apparent increase in the number of phagocytic cells in the olfactory nerve layer and glomerular layer of the mouse olfactory bulb.

During the first 14 days of the reactive stage, degenerating terminals contained swollen, distorted synaptic vesicles (Fig. 6B–D), and became much paler than nor-

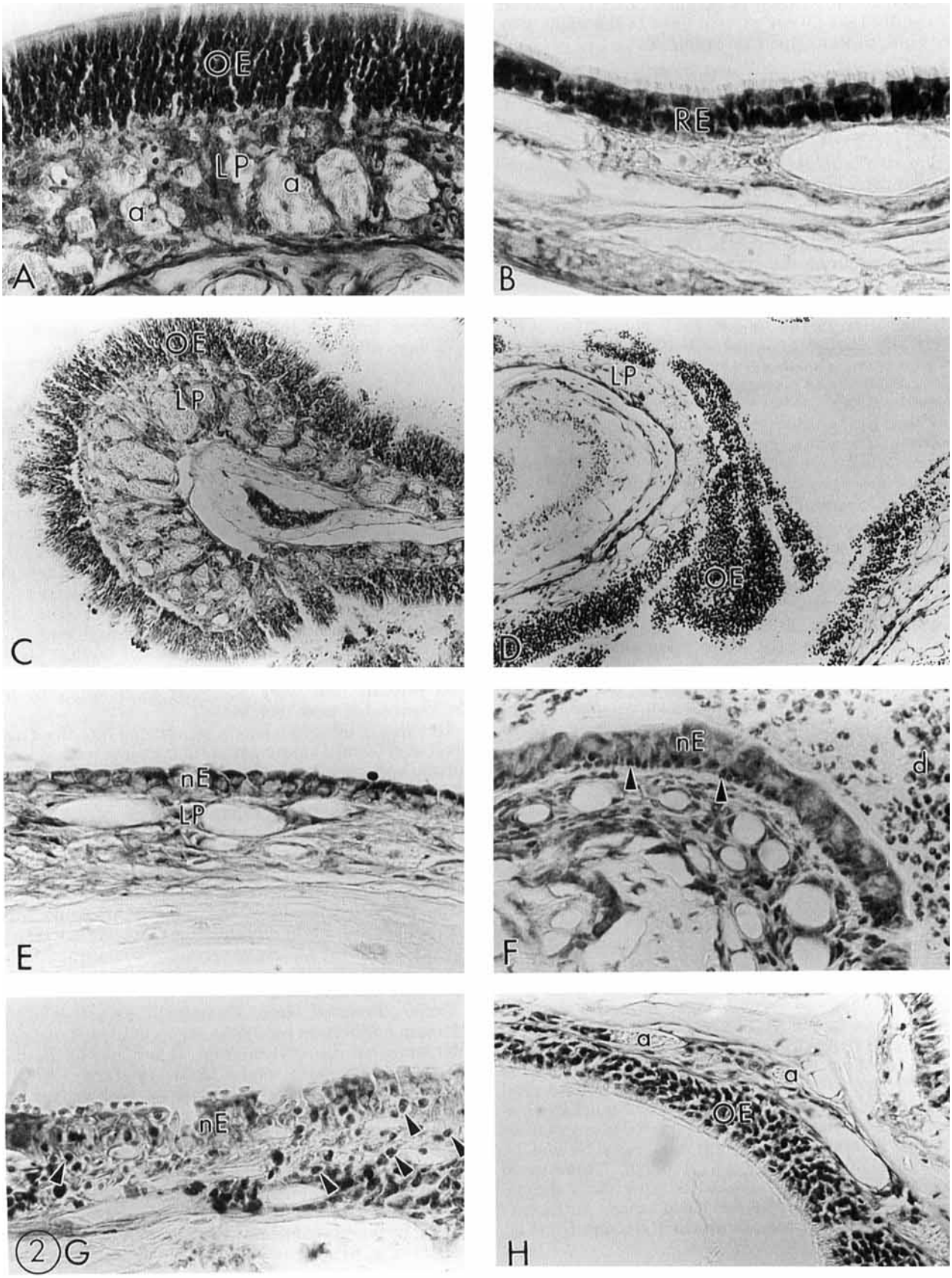

Fig. 2.

mal (Fig. 6C) or very electron dense and shrunken (Fig. 6C,D). Mitochondria were frequently disrupted (Fig. 6D). After 14 days, olfactory axons were sparse, but after 22 days, they were totally absent from the olfactory bulb.

The reactive stage was also characterized by an increase in the incidence of phagocytic cells in both the ONL and GL. At the shortest survival times (1 and 2 days), large phagocytic cells with thick and thin processes were present in the ONL. These processes frequently branched into narrow fingers of cytoplasm loosely surrounding small bundles of olfactory axons (Fig. 7A) or extending long distances through widened extracellular spaces. Within these cells were engulfed materials, lysosomes, and dense bodies (Fig. 7B). These cells had a morphological appearance similar to blood-derived macrophages (Fujita and Kitamura, 1976), but the fine processes surrounding bundles of axons suggest that they may be the ensheathing glial cells. Occasionally, cells with the morphological characteristics of microglial cells (Peters et al., 1991; Vaughn and Peters, 1968) were observed in the ONL. Large processes of these cells contained lysosomes, electron dense rings, lipid droplets, and residual bodies (Fig. 7C). In addition, astroglial processes at the border of the ONL and GL and within the GL showed phagocytosis. Astroglial processes and the perikarya and processes of phagocytic cells were more prominent within the glomeruli. Contained within these cells were remnants of degenerated terminals, lysosomes, lipid droplets, residual bodies, and electron dense rings with electron lucent centers (Fig. 7D). Due to the fairly uniform shape of the rings and the absence of membranes around them, most of the rings were probably lipid droplets. Presumably, the center consisted of saturated lipids that had been extracted by organic solvents used in the tissue preparation. Dense spheres and rings that were membrane-bound were also observed, and these were probably lysosomes. Both ring-like structures were observed frequently at survival times up to 22 days, but rarely in mice with survival times longer than 151 days. In addition, mice with short survival times (1 and 2 days) had monocyte-macrophage-like cells that appeared to be entering the GL neuropil from blood vessels (Fig. 8A).

The recovery stage, survival times longer than 25 days, was distinguished by a return of glial processes to their normal state, apparent decrease in numbers of phagocytic cells, and in some mice with survival times of 150 days or longer, reinnervation of small areas of the olfactory bulb by olfactory axons. Transsynaptic degeneration of mitral/tufted and periglomerular cell dendrites was not observed even 472 days after the lesion of the olfactory neurons. Glomerular structures were maintained even in the absence of olfactory nerve terminals. At all survival times, this neuropil contained normal dendrites and synaptic contacts of mitral/tufted and periglomerular cell dendrites (Fig. 8B). Dendrites, however, did respond to the massive degeneration in the glomeruli by engulfing debris remaining in the neuropil after the olfactory axons degenerated (Fig. 9A). Most of these dendrites were identified as mitral/tufted cell primary dendrites. Synaptic specializations also remained attached to some dendrites (Figs. 9B). These persistent specializations were opposed to glial processes, dendritic membranes, and other deafferented synaptic specializations.

Detailed observations were not made on the ultrastructure of the deeper layers of the MOB (EPL, MCL, IPL, and GRL) of experimental mice, but the structure of these layers did not appear to be affected by intranasal $ZnSO_4$ irrigation nor by degeneration of the olfactory axons. Transneuronal degeneration of neurons was not apparent. Dark neurons were occasionally observed in both control and $ZnSO_4$-treated mice, and thus, did not result from the chemical lesion of the olfactory epithelium. Dark cells observed here were likely due to removing the brain immediately after perfusion (Ebels, 1975; Stensaas et al., 1972).

In animals with long survival times (151–472 days), there were small areas of the olfactory bulb innervated by olfactory axons, while the rest of the bulb remained denervated. The innervated glomeruli were smaller than normal and were located in the superficial regions of the GL. In most of these mice, the fine structure of the olfactory axons and terminals was normal (Fig. 9C), but in one mouse (258 days survival) the axons and terminals had a more embryonic appearance and formed few synapses.

DISCUSSION

The present study confirmed the findings of others that intranasal $ZnSO_4$ irrigation results in complete degeneration of the olfactory epithelium followed with considerable delay by areas of regeneration of the sensory epithelium. These results and my fine structural analysis of terminal degeneration and regeneration in the olfactory bulb are discussed below.

Fig. 2. Epithelium in the nasal cavities of control (A, B), saline-treated (C), and $ZnSO_4$-treated (D–H) mice. **A:** Control olfactory epithelium (OE) and underlying lamina propria (LP) are illustrated. Bundles of olfactory axons (a) collect in the lamina propria. **B:** Control respiratory epithelium is shown. Respiratory epithelium (RE) consists of a single layer of columnar cells with numerous cilia and large spherical or cuboidal nuclei. **C:** Olfactory epithelium (OE) and lamina propria (LP) on the end of one turbinate in the right nasal cavity from a saline-treated mouse with 4 days survival. Note that the surface of the olfactory epithelium is mildly disrupted. **D:** Olfactory epithelium (OE) and lamina propria (LP) from the right nasal cavity of a $ZnSO_4$-treated mouse with 2 days survival. Most of the olfactory epithelium has separated from the underlying lamina propria and formed large sheets of cells. Epithelium lining the nasal cavity 4 days (**E**), 11 days (**F**), 25 days (**G**), and 151 days (**H**) after $ZnSO_4$ treatment. The new epithelium (nE) lining the septum, turbinates, and dorsal fossa above the lamina propria (LP) is first a single layer of cuboidal cells with large, pale nuclei (E). Later the new epithelium consists of two or more cell layers often with smaller cells containing dark nuclei (examples at arrowheads) located along the epithelium-lamina propria border (F). Degenerating cells (d) of the olfactory epithelium can remain in the nasal cavity for several weeks (F). In some animals (G), cells with dark nuclei (examples at arrowheads) are present in the lamina propria and appear to be entering the overlying new epithelium. In animals with long survival times after $ZnSO_4$ (H), apparently regenerated olfactory epithelium (OE) lines small areas of the right nasal cavity. Small bundles of olfactory axons (a) can often be identified beneath the regenerated olfactory epithelium. Magnifications: A, H, × 230; B, × 360; C, D, × 110; E, F, × 370; G, × 250.

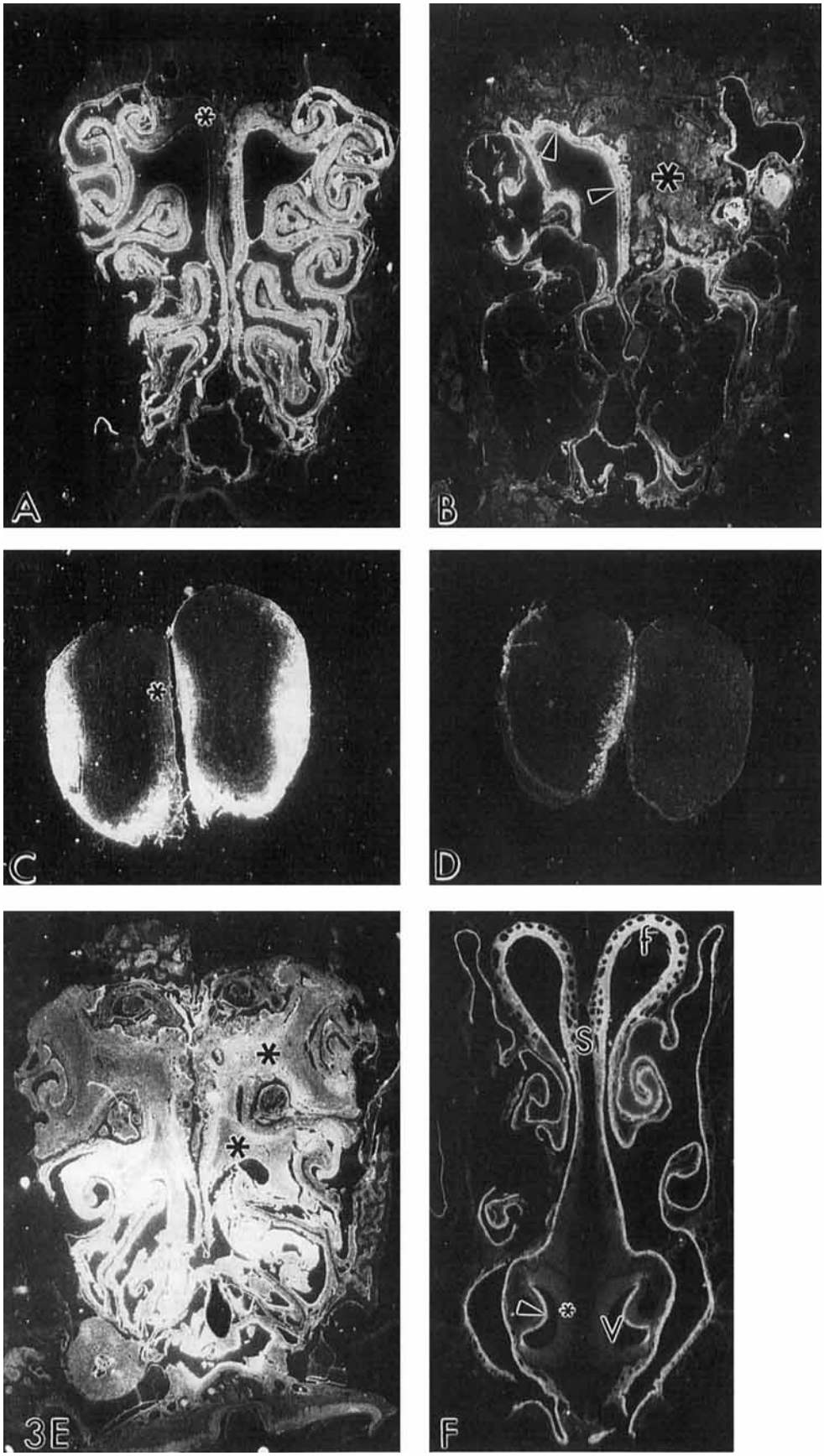

Fig. 3.

Morphological Changes in the Olfactory Epithelium

The time course and extent of degeneration and regeneration of the olfactory epithelium after intranasal administration of $ZnSO_4$ have been quite variable across a number of studies (Alberts and Galef, 1971; Cancalon, 1982; Harding et al., 1978; Matulionis, 1975, 1976; Mulvaney and Heist, 1971; Schultz, 1960; Schultz and Gebhardt, 1937, 1942; Smith, 1938, 1951). This suggests that the tissue response to $ZnSO_4$ may depend upon the animal strain (Matulionis, 1975) or species, the method of administration (Peet et al., 1937; Schultz and Gebhardt, 1938, 1942; compare Alberts and Galef [1971] with Harding et al. [1978] and Nadi et al. [1981]), the number (Schultz and Gebhardt, 1942) or duration of application(s) (Cancalon, 1982), or the concentration of $ZnSO_4$ (Schultz and Gebhardt, 1942). Despite the above, several observations exist that are similar to the results on the olfactory epithelium that are reported here. $ZnSO_4$, unlike a number of other agents that have been tested (Nadi et al., 1981; Schultz and Gebhardt, 1942), causes severe damage to the olfactory epithelium (Schultz, 1960; Schultz and Gebhardt, 1942). The damage generally consists of complete separation of the olfactory epithelium from the underlying lamina propria (Matulionis, 1976; Schultz, 1960; Schultz and Gebhardt, 1942). Within days the lamina propria is covered by a new epithelium that is morphologically quite different from the original olfactory epithelium (Matulionis, 1975, 1976; Mulvaney and Heist, 1971; Schultz, 1960; Schultz and Gebhardt, 1942; Smith, 1938). The new epithelium appears to be derived from cells in the lamina propria, possibly from the Bowman's glands (Harding et al., 1978; Matulionis, 1975, 1976; Mulvaney and Heist, 1971; Schultz and Gebhardt, 1942; Smith, 1938). The extent of regeneration usually depends upon the degree of damage induced by $ZnSO_4$. In studies where the olfactory epithelium degenerates completely, regenerated areas of olfactory epithelium are observed only after one to several months (Harding et al., 1978; Matulionis, 1975; Mulvaney and Heist, 1971; Schultz, 1960).

In the present study, the absence of ^{3}H-amino acid labelling in the olfactory bulb of $ZnSO_4$ treated animals with short survival times (4–11 days) suggested that olfactory axons no longer contacted the olfactory bulb. It is possible, however, that cells from the degenerating olfactory epithelium and infiltrating macrophages that filled the nasal cavity at these times prevented tritiated amino acids from reaching areas of the olfactory epithelium that might have remained. This could have occurred in animals with longer survivals (49–595 days) after $ZnSO_4$ treatment as well because some of these animals had nasal capsules that were very distorted by necrotic damage to the lamina propria and underlying cartilage and bone. Examination of serial sections through the nasal capsule, however, did not yield patches of epithelium that were initially spared the $ZnSO_4$ lesion or that were unlabelled in animals with long survival times.

Behavioral Studies

Behavioral recovery after intranasal administration of $ZnSO_4$ can occur as early as 5–10 days (Alberts and Galef, 1971; Slotnick and Gutman, 1977), within 6 or 7 weeks (Harding et al., 1978; Vandenbergh, 1973), or after several months (Harding et al., 1978), and the recovery rate depends upon various factors that are outlined above. As in the present study, Harding and coworkers (1978) found that behavioral recovery after intranasal $ZnSO_4$ irrigation was correlated with regeneration of olfactory epithelium and olfactory axon connections with the olfactory bulb, and that regeneration of only a small percentage of the normal olfactory epithelium was sufficient to produce behavioral recovery.

Morphological Changes in the Olfactory Bulb: Reactive and Recovery Stages

Few investigators have examined the morphological changes occurring in the olfactory bulb after intranasal $ZnSO_4$ irrigation. Alberts and Galef (1971) report that the olfactory bulb is unchanged 1 week after $ZnSO_4$ administration. This was not true in the present study. Disruption of the olfactory nerve layer was observed as early as 1 day after the treatment, and proceeded to worsen with time. The difference between the results presented here and those of Alberts and Galef (1971) is most likely related to the relative damage to the olfactory epithelium induced by $ZnSO_4$ in the two studies. Alberts and Galef (1971) reported only temporary behavioral deficits, while the deficits observed in my study were complete and long lasting. On the other hand, the light microscopic results shown in the present study are in agreement with Harding and coworkers (1978), who used an irrigation method identical to that reported here, and who showed that olfactory axons were absent from the olfactory bulb at 3 weeks and that there was regeneration of olfactory axons to some areas of the olfactory bulb 1 year following $ZnSO_4$ irrigation. Our experiments using transport of tritiated amino acids also show that small areas of sen-

Fig. 3. Darkfield photomicrographs of coronal sections of nasal cavities and olfactory bulbs from animals treated intranasally with ^{3}H-amino acids. The nasal cavity in **A** is from a control mouse. ^{3}H-amino acids administered to the right nasal cavity occasionally did not label areas of the olfactory epithelium in the left dorsal fossa (*) and septum. The olfactory bulb from the animal shown in A is illustrated in **C**. Note that the medial surface of the left olfactory bulb (*) is only lightly labelled. The silver grains (represented by the white areas) are restricted to the olfactory nerve and glomerular layers; the dorsal surface of the olfactory bulb at this level is devoid of olfactory axon projections. Figures **B** and **D** are from a $ZnSO_4$-treated animal with 593 days survival. This animal has olfactory epithelium lining the left dorsal fossa and septum (arrowheads) and small areas along the lateral edge of the nasal cavity. These areas project to the medial surface and dorsal lateral border of the left olfactory bulb (D). Note that the area of labelling in the bulb is thinner and less dense than that of the control animal shown in C. The right nasal cavity from this animal is also highly disrupted (*) and calcified (B). At short survival times after $ZnSO_4$ irrigation (11 days; **E**), the nasal cavity is filled with degenerating cells (and possibly macrophages) that take up the radioactive label (*). In **F**, it is apparent that the ^{3}H-amino acids label the olfactory epithelium in the dorsal fossa (f) and along the septum (S) and the respiratory epithelium of the main region of the nasal cavity and vomeronasal organ (arrowhead), but do not label the sensory epithelium (*) of the vomeronasal organ (V). Magnifications: A, B, E, × 14; C, F, × 17; D, × 18.

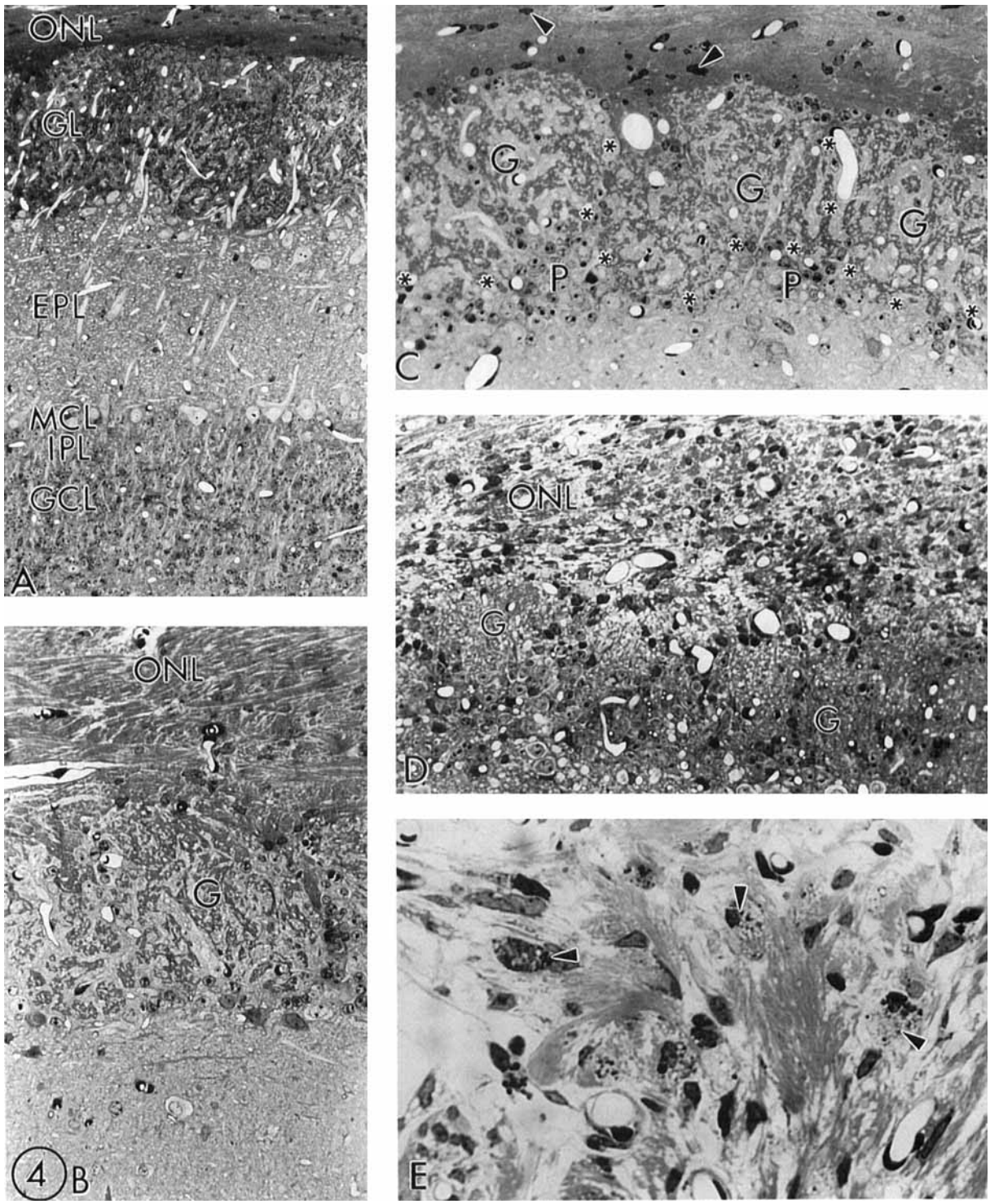

Fig. 4. Light micrographs of the olfactory bulb. **A, C:** Control olfactory bulbs. The olfactory bulb is a laminated structure (A) consisting of the following layers: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), and granule cell layer (GCL). Darkly stained olfactory axons enter the glomeruli and stand out from the more lightly stained dendrites in the glomeruli (G) in C. Glomeruli are somewhat separated by periglomerular regions (P) and loosely defined in this micrograph by the asterisks (*). Glial cells of the olfactory nerve layer are identified at the arrowheads. **B:** Olfactory nerve and glomerular layers from an animal that survived 8 days after intranasal irrigation with saline. Note that the bundles of olfactory nerves in the olfactory nerve layer (ONL) are somewhat separated, relative to that of the control animal in C, but no major disruption is apparent in the glomeruli (G). **D, E:** Olfactory bulbs from $ZnSO_4$-treated mice with 6 days survival and 8 days survival, respectively. Major disruption is apparent in the olfactory nerve layer (ONL) and glomeruli (G). In E, phagocytotic cells with engulfed debris are shown (examples at arrowheads). Magnifications: A, × 170; B, D, × 360; C, × 300; E, × 590.

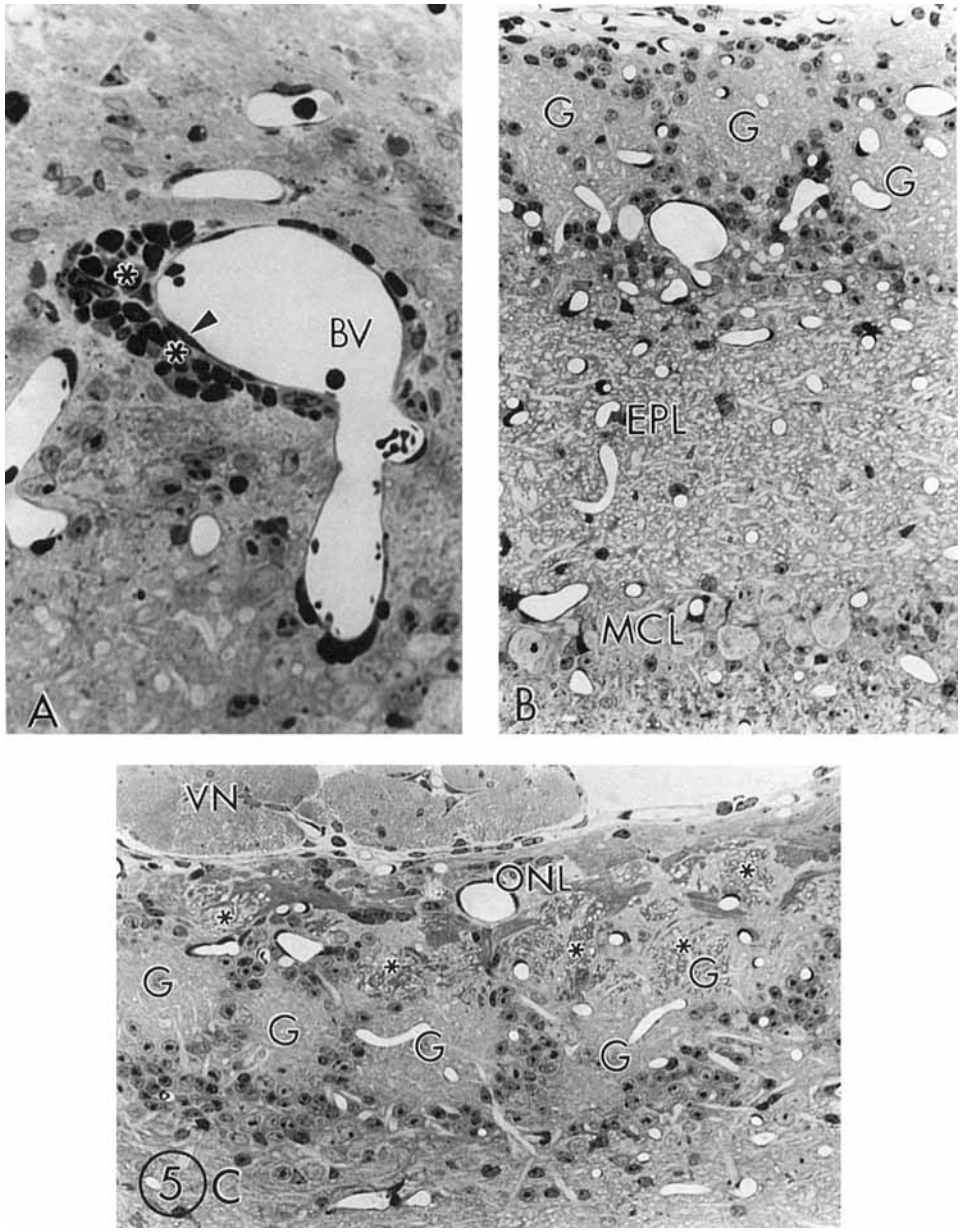

Fig. 5. A blood vessel at the border between the olfactory nerve layer and the glomerular layer from a $ZnSO_4$-treated mouse with 2 days survival (**A**). Numerous darkly stained cells (*) are gathered adjacent to this blood vessel (BV), but on the brain side of the endothelial cells (example at arrowhead). Twenty-two days after intranasal $ZnSO_4$ irrigation, the reactive response of the olfactory bulb to degenerating olfactory axons appears to be over (**B**). The olfactory nerve layer is gone, reactive phagocytic cells and glia are not observed in the glomeruli (G), olfactory axons are missing from the glomeruli, and the external plexiform (EPL) and mitral cell (MCL) layers appear normal. With increasing survival time, as shown in **C** 151 days after the $ZnSO_4$ lesion, olfactory axons reinnervate the olfactory bulb. This micrograph of the medial, right olfactory bulb illustrates a normal vomeronasal nerve (VN), an olfactory nerve layer (ONL) that is thinner than normal, and olfactory axons entering superficial (*) glomeruli (G), but not penetrating to deeper regions of the glomerular layer. Magnifications: A, × 590; B, C, × 230.

sory epithelium regenerated in mice with long survival periods after the lesion.

Reactive Stage. Following intranasal $ZnSO_4$ administration, all olfactory axon terminals degenerated; however, they did so at different rates. The rate of degeneration observed here is comparable to that observed by Pinching and Powell (1972) in the rat olfactory bulb after surgical destruction of the olfactory epithelium. Olfactory terminals, however, degenerate more rapidly after intracranial olfactory nerve transections; nearly all of the terminals are engulfed by glia 3 days after nerve transection in the rat (Graziadei and

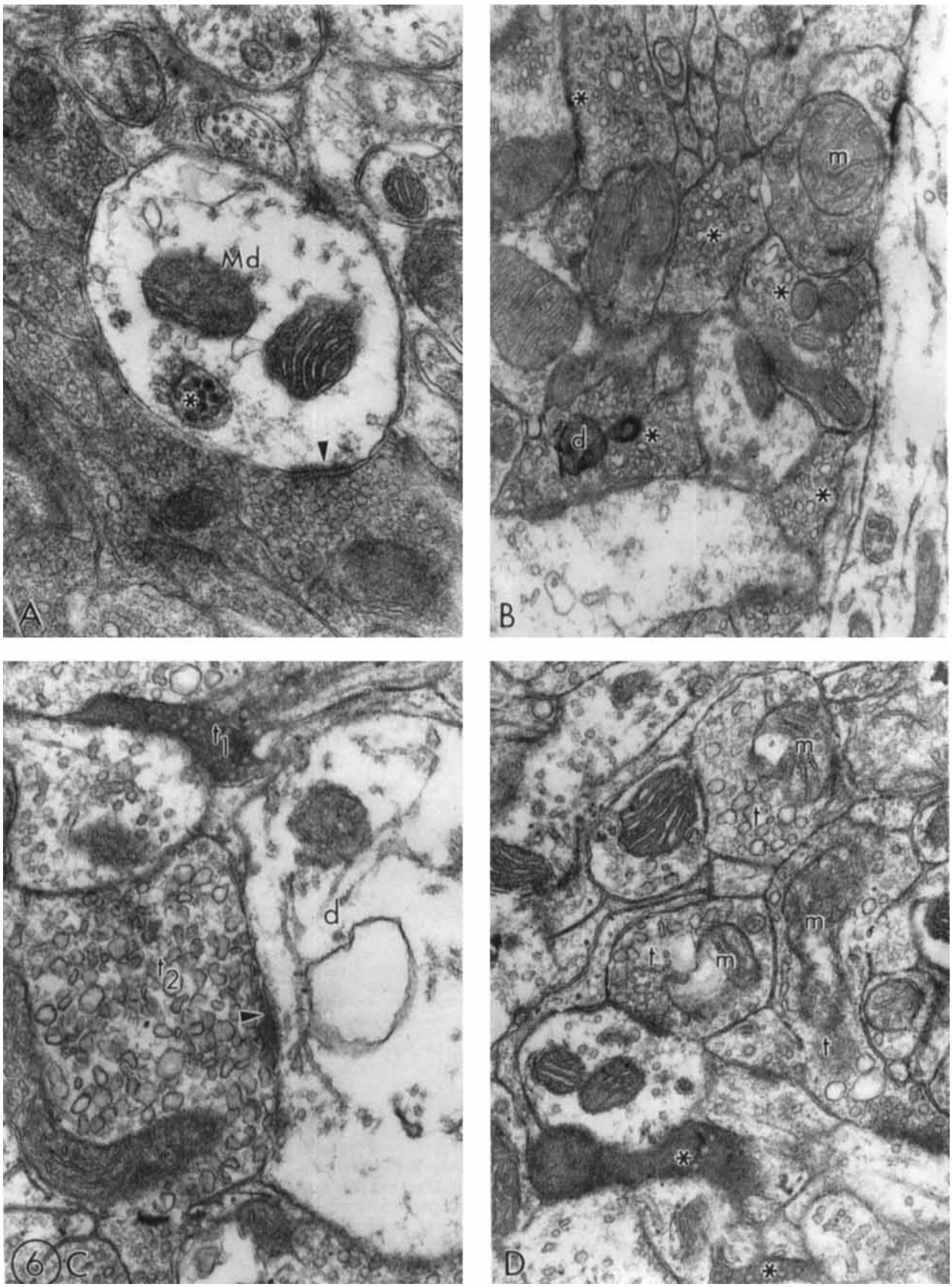

Fig. 6.

Monti Graziadei, 1980). The differences in the rates of degeneration in these studies may be due to the site of the lesion. The nerve transections were performed very close to the terminal end of the olfactory axons while the chemical and surgical destruction of the olfactory epithelium affected the olfactory neurons at the level of the cell body. This hypothesis is supported by the observation (Graziadei and Monti Graziadei, 1980) that olfactory nerve terminals in the rostral olfactory bulb degenerate more rapidly than those in the caudal olfactory bulb.

The two types of degenerating olfactory axon terminals—pale, swollen terminals and dark, shrunken terminals—that were observed in the present study were seen in previous studies that used a surgical lesion to deafferent the olfactory bulb (Graziadei and Monti Graziadei, 1980; Pinching and Powell, 1972). It was proposed that terminals with these morphologies represent different types of degeneration (Graziadei and Monti Graziadei, 1980) or different stages of degeneration (Pinching and Powell, 1972). This later hypothesis (i.e., that pale terminals with swollen, distorted synaptic vesicles ultimately become dark, shrunken terminals) was suggested by Kawana and colleagues (1971) for degenerating terminals in the caudate nucleus. In other systems, however, it was proposed that these different terminal morphologies represent different types of degeneration (Mugnaini and Friedrich, 1981; Rustioni and Sotelo, 1974b). Since both types of degenerating terminals coexisted in our study, it was not possible to determine whether these different morphologies represent different types of terminal degeneration or different stages within the same degenerative process.

Engulfment by astroglial processes was an important mechanism for removing degenerating olfactory axon terminals and debris from the mouse olfactory bulb after intranasal $ZnSO_4$ irrigation. Similar results have been reported after surgical lesions of the olfactory neurons (Doucette et al., 1983; Graziadei and Monti Graziadei, 1980; Pinching and Powell, 1972). In addition, phagocytic cells identified as microglial cells occur in the rat olfactory bulb following olfactory nerve transection (Doucette et al., 1983) and in the mouse bulb after intranasal irrigation with $ZnSO_4$ as shown here. Phagocytic cells have also been described in several other principal sensory nuclei after lesions of the primary afferent fibers (Gentschev and Sotelo, 1973; Rustioni and Sotelo, 1974b; Vaughn and Pease, 1970; Wong-Riley, 1972).

The origin of phagocytic cells in the CNS has been studied after traumatic injuries (Adrian and Williams, 1973; Blakemore, 1972; del Cerro and Monjan, 1979; Konigsmark and Sidman, 1963), infections (del Cerro et al., 1975), and non-traumatic lesions (Matthews and Kruger, 1973a,b; Vaughn et al., 1970). These cells may be blood-borne (hematogenic) or endogenous in adulthood (microglial cells). Since morphological criteria alone may not be sufficient to identify the origins of phagocytic cells (Fujita and Kitamura, 1976), vascular injections of radioactive thymidine (Fujita and Kitamura, 1976; Konigsmark and Sidman, 1963) or colloidal carbon (del Cerro and Monjan, 1979) has been used to label those cells of hematogenic origin. Using this procedure, Fujita and Kitamura (1976) showed that phagocytic cells in the facial nucleus during retrograde degeneration of the facial nerve had a hematogenic origin.

Considering the difficulty in identifying the phagocytic cells based only on their morphology, it was not surprising that the origin of all the phagocytic cells in the olfactory bulb following intranasal $ZnSO_4$ irrigation was difficult to determine. Cells fitting the description of microglial cells (Peters et al., 1991) were present in the olfactory bulb of control animals. Similar cells, but with increased engulfed material, were more frequently observed after the lesion. The presence of monocytes/macrophages in the space between the endothelial cells and the basement membrane supports a hematogenous origin for at least some of these phagocytic cells.

Following $ZnSO_4$-induced terminal degeneration, dendrites in the GL of the mouse olfactory bulb participated in the removal of debris and portions of terminal membranes. In several other studies on degeneration of olfactory nerve terminals (Estable-Puig and DeEstable, 1969; Graziadei and Monti Graziadei, 1980; Harding et al., 1977; Pinching and Powell, 1972) this phagocytic activity by dendrites was not reported, however, it was observed in the rat olfactory bulb by Doucette and colleagues (1983) and in the rabbit bulb by Berger (1973). Phagocytic activity by dendrites has also been reported in several other brain regions (Gentschev and Sotelo, 1973; Ghetti et al., 1975; Rustioni and Sotelo, 1974a; Walberg, 1963; Wiśniewski et al., 1972), and therefore is not an activity specifically related to the olfactory bulb or to chemical lesions.

Transneuronal degeneration has been reported in the rabbit (Berger, 1973; Matthews and Powell, 1962; Pinching and Powell, 1971a) and the rat (Pinching and Powell, 1971a) olfactory system after surgical destruction of the olfactory epithelium. There is marked species variability in this form of degeneration (Pinching and Powell, 1971a). In the rabbit, within 10 days after the lesion some of each type of bulbar neuron showed transneuronal degenerative changes that usually included increased cytoplasmic density. In the rat, transneuronal degeneration was not observed until 200 days after the lesion, and then changes were present only in a small number of glomeruli. It is possible that the

Fig. 6. **A:** Mitral or tufted cell dendrite (Md) that receives synaptic input (arrowhead) from one olfactory axon terminal. A multivesicular body (*) is present in this dendrite. Olfactory axon terminals 1 day (B) and 11 days (C and D) after intranasal $ZnSO_4$ irrigation. **B:** The earliest changes observed in the olfactory axon terminals (*) include swollen, distorted synaptic vesicles and swollen mitochondria (m). One terminal shown here also contains some dense debris (d). In **C**, dense, shrunken terminals (t_1) are observed in the same area as large, pale olfactory axon terminals (t_2) containing swollen and distorted synaptic vesicles. The pale terminal forms a synaptic contact (arrowhead) on a dendrite (d). **D:** At 11 days, some terminals are shrunken and very electron dense (*), while other terminals (t) have disrupted mitochondria (m) and continue to have swollen, distorted synaptic vesicles. Magnifications: A, × 39,600; B, × 38,000; C, × 49,200; D, × 43,100.

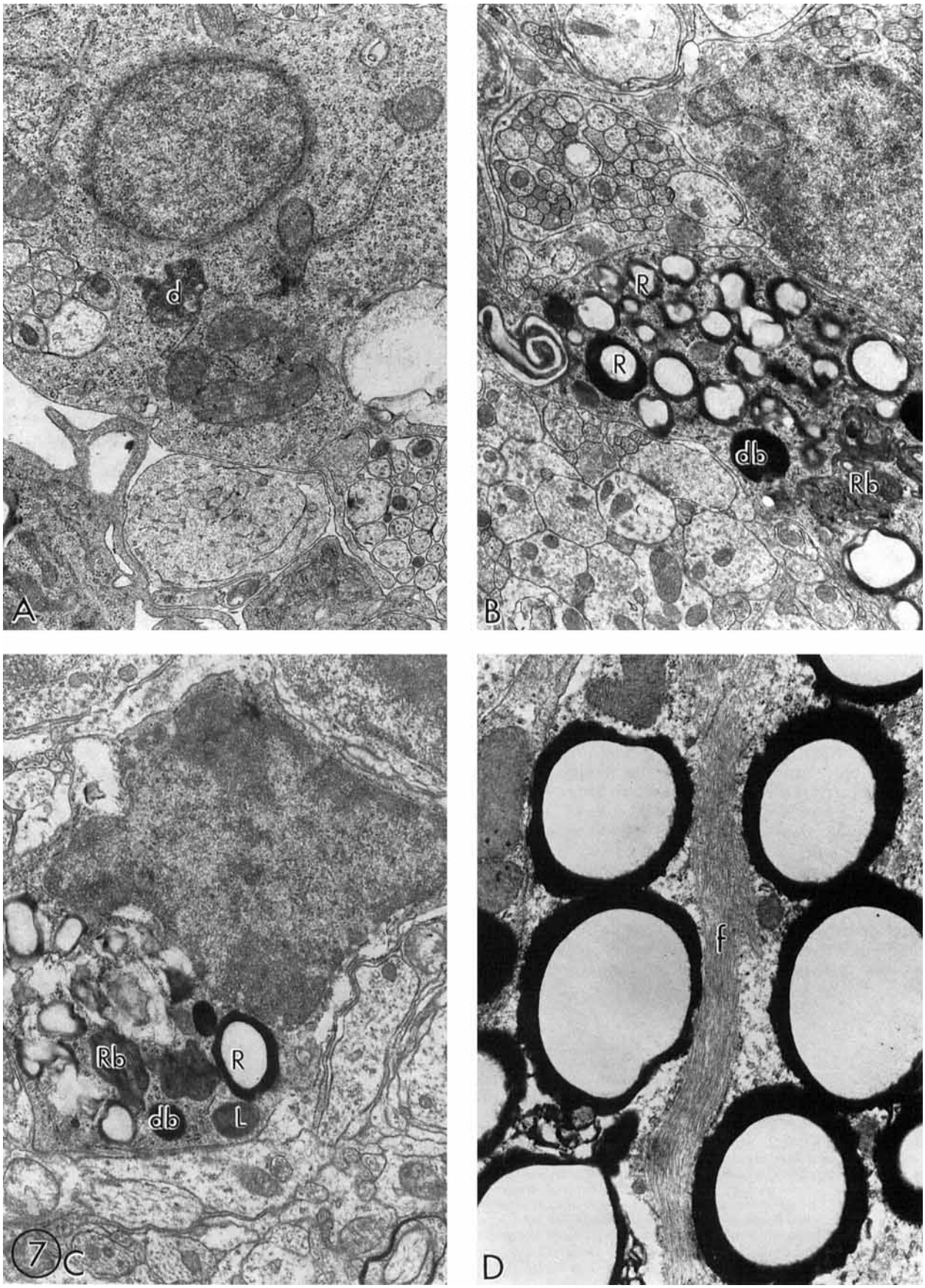

Fig. 7. Response of glial cells and phagocytic cells in the olfactory bulb to the $ZnSO_4$ lesion. Two days (**A**) and 5 days (**B**) after the lesion, there are numerous phagocytic cells in the olfactory nerve layer (A) and at the border of the glomerular layer (B) that contain various amounts of debris (d), dense bodies (db), residual bodies (Rb), and dense rings (R). A microglial cell in the periglomerular region (**C**) contains a lysosome (L), dense body (db), residual body (Rb), and dense ring (R) 11 days after the lesion. Astrocytes (**D**) are also phagocytic, and this astrocyte contains large, dense rings and a thick bundle of glial filaments (f) 22 days after $ZnSO_4$ treatment. Magnifications: A, × 18,700; B, × 11,100; C, × 8,500; D, × 33,900.

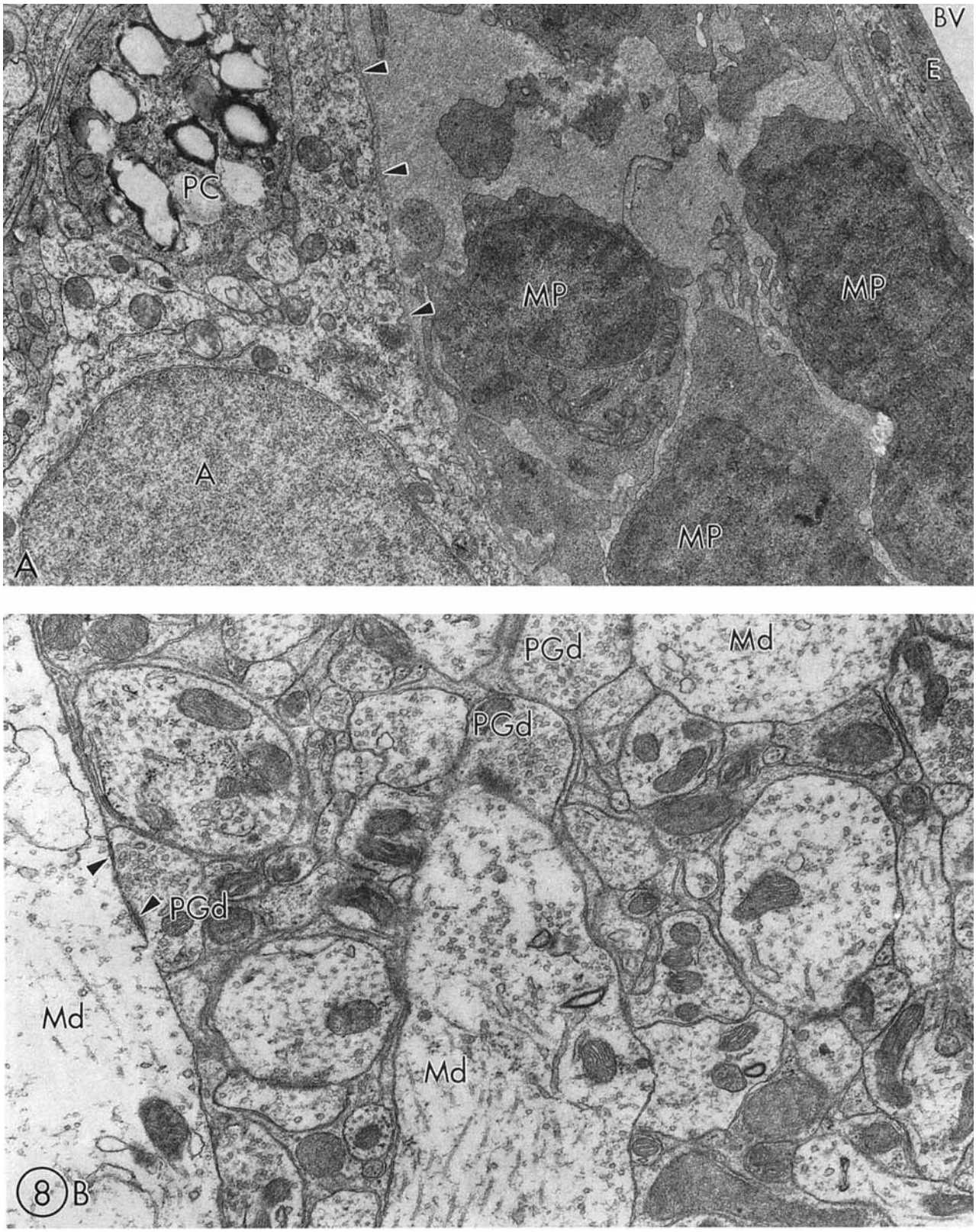

Fig. 8. **A:** Hematogenic origin of some of the phagocytic cells in the olfactory bulb 2 days after intranasal $ZnSO_4$ irrigation is suggested by the presence of monocytes or macrophages (MP) in the space between the endothelial cell (E) lining this blood vessel (BV) and the basal lamina of the vessel (arrowheads). An astrocyte (A) and a phagocytic cell (PC) containing evidence of phagocytic activity are present on the brain side of the basal lamina. **B:** Four hundred seventy-two days after the lesion, most of the glomeruli are still not innervated by olfactory axons. In these glomeruli, however, the normal dendro-dendritic synaptic contacts are observed between mitral/tufted cell dendrites (Md) and periglomerular cell dendrites (PGd). A reciprocal synapse is indicated at the arrowheads. Magnifications: A, × 5,600; B, × 23,400.

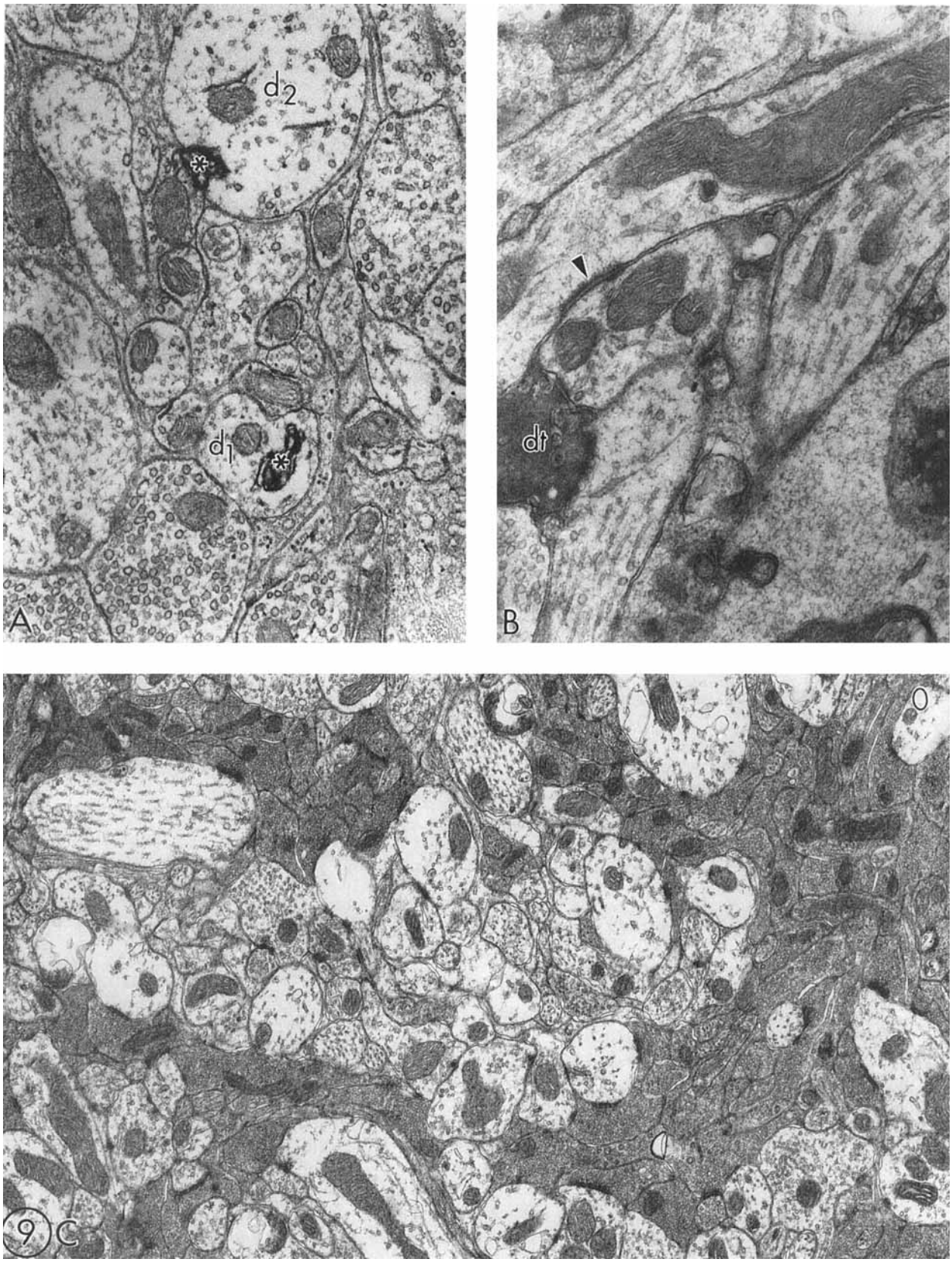

Fig. 9. **A:** Dendrites in the olfactory bulb following $ZnSO_4$-treatment contain (d_1) and appear to engulf (d_2) dense, membranous debris (*); 22 days after the lesion. **B:** Denervated postsynaptic sites (arrowhead) are observed along an unidentified dendrite 51 days after the lesion; similar denervated junctions were observed in the rat bulb (Pinching, 1969). B also contains a dense, degenerating terminal (dt). **C:** With long survival times after the $ZnSO_4$ lesion, some areas of the glomerular layer appear normal and contain regenerated olfactory axons and terminals (415 days). Magnifications: A, × 33,300; B, × 34,600; C, × 16,600.

changes observed in the rat are related more to an aging process rather than a result of the lesion.

While neurons do not appear to degenerate in the rat bulb, dendrites in the glomeruli do show transient (3–10 days) swelling after the lesion (Graziadei and Monti Graziadei, 1980). Transneuronal degeneration and swollen dendrites were not apparent in the mouse olfactory bulb after intranasal $ZnSO_4$ irrigation. Perhaps dendrites were swollen in the rat bulb after olfactory nerve transection (Graziadei and Monti Graziadei, 1980) because they were rapidly deafferented, while after $ZnSO_4$ administration the deafferentation is a more gradual process.

Recovery Stage. In the present study, the recovery stage was characterized by glomeruli that were smaller than during the reactive stage or than in control animals due to the loss of the olfactory axons and shrinking of the glial processes back to their normal size. While numbers of phagocytic cells within the glomeruli were not counted, there appeared to be fewer of these cells during the recovery stage than during the reactive stage. The method of removal of these cells is not known, but it is possible that they exit the CNS via the vascular system.

Reinnervation of small areas of the bulb by olfactory axons was another feature common to late stages of the recovery period. Reinnervation by olfactory axons occurred much later (after 150 days) following $ZnSO_4$ treatment than after surgical transection of the olfactory axons (24–42 days [Doucette et al., 1983; Graziadei and Monti Graziadei, 1980]). The appearance of the glomeruli and the olfactory axons in mice with survivals of 150 days or greater suggested regrowth rather than an initial sparing of the axons from the $ZnSO_4$ lesion. The ONL was thinner than normal and the olfactory axons usually only penetrated to the more superficial regions of the glomeruli. As following surgical lesions, the fine structure of regenerated olfactory axons and terminals was identical to that of control mice.

The processes of olfactory receptor cell turnover or renewal (Andres, 1975; Farbman, 1990; Graziadei and Metcalf, 1971; Moulton and Fink, 1972; Moulton et al., 1970; Nagahara, 1940; Yamamoto et al., 1976) and olfactory receptor cell replacement after cell death induced by transection of the olfactory axons (Graziadei and Monti Graziadei, 1978, 1980; Harding et al., 1977) have been widely reported (also see Breipohl et al., 1986). In the rodent olfactory bulb after transection of the olfactory axons, the olfactory axons rapidly degenerate and by 10 days after the lesion, they have been removed by astroglial cells (Graziadei and Monti Graziadei, 1980). By 24–30 days after this lesion, olfactory axons are again present in the olfactory bulb and the ultrastructure of the glomeruli returns to normal within 6 weeks (Doucette et al., 1983; Graziadei and Monti Graziadei, 1980).

Regeneration of the olfactory epithelium to its normal structure is much less extensive and significantly slower after intranasal $ZnSO_4$ administration than following olfactory axon lesions. Presumably, this is due to destruction of immature neurons and basal cells by $ZnSO_4$. Regeneration of the epithelium following $ZnSO_4$ administration varies from as early as 30 days (Mulvaney and Heist, 1971) to as late as 6 months (Schultz, 1960) after the lesion. Using the procedure developed by Margolis, regeneration of the olfactory epithelium does not always occur (Margolis et al., 1974), but some regeneration of the olfactory epithelium has been reported (Harding et al., 1978). Regeneration of portions of the olfactory epithelium at 1 year after the lesion coincided with the presence of olfactory axons in glomeruli of the anterior portion of the MOB (Harding et al., 1978). These findings support the hypothesis given here that olfactory axons present in the olfactory bulb of mice with survival times after intranasal $ZnSO_4$ irrigation of 150 days or more are the products of a regenerated olfactory epithelium. The present study demonstrated that, while the fine structure of the reinnervated areas of olfactory glomeruli returns to normal after $ZnSO_4$ lesions, most of the olfactory bulb is devoid of sensory innervation.

ACKNOWLEDGMENTS

I am thankful for advice and financial assistance from Dr. Aldo Rustioni during much of the research that went into this paper, for scientific and technical discussions with Drs. Frank L. Margolis and Thomas V. Getchell, and for helpful suggestions on the manuscript from Dr. Leslie Tolbert and two anonymous reviewers.

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