Chem. Senses 27: 623–628, 2002

Biopsies of Human Olfactory Epithelium

B.W. Jafek, B. Murrow, R. Michaels, D. Restrepo and M. Linschoten

Rocky Mountain Taste and Smell Center, Denver, CO, USA

Correspondence to be sent to: Bruce W. Jafek, Department of Otolaryngology–Head and Neck Surgery, UCHSC (B-205), 4200 East Ninth Avenue, Denver, CO 80262, USA. e-mail: bruce.jafek@uchsc.edu

Abstract

It has been shown that olfactory epithelium can be safely biopsied from the living, intact human being. Observations of the ultrastructure of this epithelium shows changes that can then be correlated with the etiology and degree of olfactory loss, allowing a greater understanding of both normal transduction and of the pathology of dysfunction. Examples of the common forms of olfactory dysfunction are presented and discussed. Additionally, the technique will allow additional immunohistochemical and molecular study of the tissue, will increase the understanding of both normal and pathological function and should translate to new therapeutic regimens.

Introduction

Biopsy of olfactory epithelium in the intact human being is essential in understanding human disease and dysfunction, as well as in obtaining 'normal' epithelium for research. In 1982 we developed an instrument and technique for the safe biopsy of the olfactory epithelium in the living, intact human being (Lovell *et al*., 1982). Since that time, the technique has been in continuous use in our laboratory and disseminated to a number of centers in the USA, as well as abroad. Major complications have not occurred and the biopsy technique has continued to safely and reliably provide tissue for study.

Preliminary evaluation

Prior to biopsy, the patient with a chemosensory complaint undergoes detailed historical and physical evaluation. This evaluation usually focuses attention upon one of the >200 conditions and an equal number of putative medical reactions or toxicities that have been linked to olfactory loss (Schiffman, 1983; Hastings and Miller, 1997). We use a standardized historical review, followed by a detailed otolaryngological examination and neurological and general examination as indicated by the initial evaluation. Our psychophysical testing includes a butyl alcohol threshold test (Linschoten *et al*., 2001), a seven item smell identification test (Cain *et al.*, 1988), the University of Pennsylvania Smell Identification Test (UPSIT) (Doty *et al.*, 1984) and taste testing as indicated. The details of the testing have been published previously (Hill and Jafek, 1989). Radiological evaluation of the olfactory system is limited to coronally oriented computerized tomography of the ethmoid region. For additional evaluation, high resolution coronal MRI images, with and without contrast material and with fat suppression techniques, offer the best opportunity to study the olfactory bulbs and tracts (Truit and Kelly, 1993). Other routine tests (e.g. CBC and blood chemistries) rarely produce diagnostic results in olfactory dysfunction and are not obtained routinely. Additional considerations and an algorithm for the evaluation of patients with loss of smell have recently been presented (Jafek *et al*., 2000).

Method

The patient is placed in the supine position and the operating microscope is used to evaluate the internal nose. Alternatively, the endoscope might be used, but the operating microscope, with a self-retaining speculum, stabilized in the non-dominant hand, offers maximal exposure and three-dimensional visualization with variable magnification and is preferred by the authors (Lovell *et al*., 1982).

A 4% cocaine nasal spray is used for vasoconstriction and anesthesia. Alternatively, a combination of 1% lidocaine and 0.05% oxymetazoline might be used. Following an initial nasal spray, a cotton pledget soaked in the mixture is inserted superiorly into the olfactory cleft until resistance is encountered to maximize vasoconstriction and anesthesia. The biopsy is obtained from the anterior septum, as high as possible, just anterior and superior to the insertion of the middle turbinate. The olfactory biopsy instrument is a hook-shaped open tool that is designed to obtain the biopsy as free of crush artifact as is possible. The instrument (SP7-31609) is commercially available by custom order through the Bausch & Lomb Surgical/Chiron Vision/Storz Instrument Co. (Manchester, MO). Following placement in the superior nasal vestibule, the cutting edge of the biopsy

© Oxford University Press 2002. All rights reserved.

instrument is rotated 90º and gently pressed against the septal mucosa. With a light downward pull, the biopsy is obtained and the instrument withdrawn (Figure 1). Patients are advised not to blow their nose for 24 h and to avoid heavy lifting or straining after the biopsy. Biopsies are obtained from the flat nasal septum, rather than the more convoluted lateral superior turbinate. Olfaction is not adversely affected by biopsy (Lanza *et al*., 1994).

Alternatively, Lanza and Trojanowski have described the use of cupped forceps to obtain olfactory tissue (Trojanowski *et al*., 1991; Lanza *et al*., 1993). The advantage of the forceps, regardless of the design, is that it minimizes the occasional loss of the specimen and can obtain a larger specimen. The disadvantage is that it induces additional trauma and a 'crush artifact' to the specimen. Monti-Bloch *et al*. (Monti-Bloch *et al*., 1998) recommended the use of a bristle brush to obtain nasal chemosensory tissue, but this has not worked well in our hands. Hasegawa *et al*. (Hasegawa *et al*., 1986) also devised their own biopsy instrument.

Specimens may be processed for routine electron microscopic evaluation of the epithelial fine structure (Jafek *et al*., 1997), for immunohistochemical study (Jafek *et al*., 1997) or for single cell study or recording (Murrow *et al*., 2000).

Microscopic structure of the human nasal mucosa

Six types of epithelium line the nasal passages in humans. Anteriorly, in direct contact with the environment, there is a keratinized, stratified squamous epithelium. Anterio-laterally, the sinus cavities are lined by low pseudostratified respiratory epithelium. Postero-laterally, there is an abrupt transition to a moist, non-keratinized stratified epithelium. Superiorly, there is a pseudostratified columnar respiratory epithelium which interdigitates with and, more superiorly, transitions to the ciliated columnar olfactory epithelium (Jafek, 1983). About 1 cm posterior to the anterior columella and 0.3–0.4 cm above the floor of the nose lies the organ of Jacobson (VNO) with its unique ciliated epithelium (Moran *et al*., 1991; Jafek *et al*., 1997; Knecht *et al*., 2001). This organ processes pheromones in lower vertebrates and is thought by some to function similarly in humans (Jafek *et al*., 1997). Others are less confident that the human VNO is functional (Meredith, 2001).

Olfactory epithelium

The human olfactory epithelium is a pseudostratified columnar epithelium that rests on a highly cellular lamina propria that contains the Bowman's glands and extends ~150 μm down to the underlying bone or cartilage. It

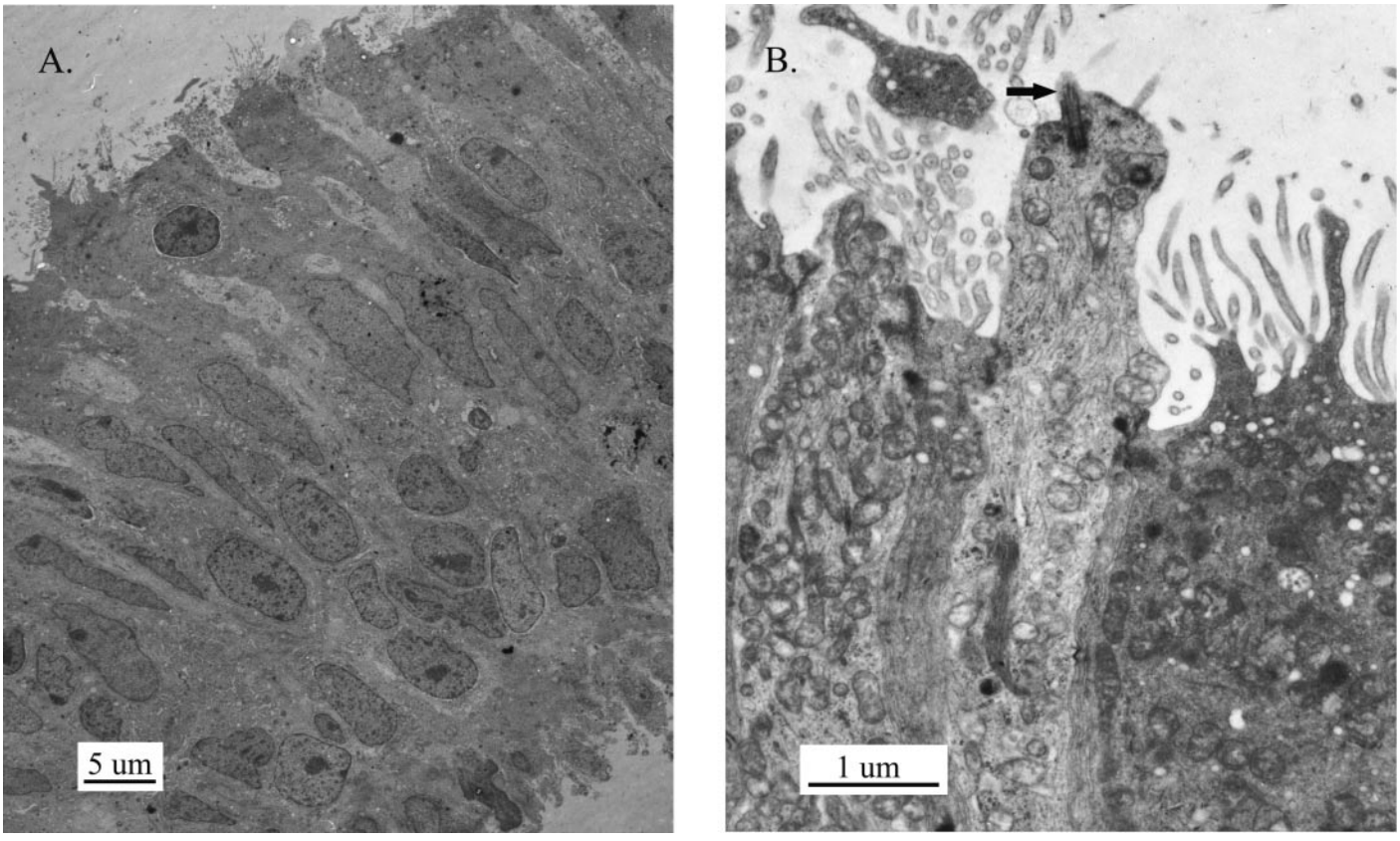

Figure 1 **(A)** Low power transmission electron micrograph (TEM) of a sheet of olfactory epithelium obtained with the biopsy technique. **(B)** Higher power electron micrograph of apical surface of olfactory epithelium. An olfactory vesicle exhibits a basal body extending into an olfactory cilium (arrow).

contains four major cell types, ciliated bipolar olfactory receptors, microvillar cells, sustentacular cells and basal cells. All except the basal cells project to the surface. In addition, occasional degenerating cells and inflammatory cells, primarily lymphocytes, are seen (Meredith, 2001).

Pathology

The histopathology of the dysfunctional olfactory epithelium confirms the hypothesis that olfactory dysfunction is usually accompanied by ultrastructural change that can be correlated with the nature and degree of dysfunction (Jafek *et al.*, 1997). Over 200 conditions and an equal number of medications and toxins have been associated with olfactory loss, although many of these reports are anecdotal and poorly documented. Schiffman (Schiffman, 1983) and Amoore (Amoore, 1986) produced comprehensive early reviews, which were subsequently updated and reorganized by Hastings and Miller (Hastings and Miller, 1997), focusing primarily on the 'toxic' exposures. Others (Doty *et al.*, 1991; Yamagishi and Nakano, 1992; Seiden, 1997a,b) offer more recent reviews.

Four etiologies of olfactory loss (post-traumatic, post-viral, nasal/sinus disease and idiopathic) constitute 70–85% of reported cases (Seiden, 1997a,b). The histopathology, as elucidated by olfactory biopsy, is as follows.

Post-traumatic, or 'post-concussive', olfactory disorder (PTOD) is found in ~5% of head injury patients (Jafek *et al.*, 1989). Occipital trauma is most common with injury or laceration of the 'tethered' fila olfactoria. Frontal impacts produced less dysfunction than back or side impacts (Doty *et al.*, 1997). Intracranial hemorrhage is seen in more severe cases. In our observations, the pathology of the olfactory epithelium in PTOD consisted of three principal changes. First, the general epithelial orientation was disorganized. The epithelium appeared thicker as individual cells were enlarged or appeared degenerate. The nuclei, which are normally arranged in a band across the middle of the epithelium, were dispersed throughout the epithelium and even approached the epithelial surface. Second, axon proliferation was observed, especially just below the basement membrane, but even throughout the epithelium. Third, the olfactory receptors were diminished in number. Those that were present were often found to be 'bald', lacking olfactory cilia projecting from their dendritic vesicles (Figure 2). Occasionally, basal bodies were seen within the vesicles, but these were diminished and appeared unconnected to projecting cilia (Jafek *et al.*, 1989). These three consistent observations, olfactory disruption, axon proliferation and absence of ciliogenesis, supported severance of the fila olfactoria at the cribiform plate as the mechanism of injury in PTOD. Others confirmed these observations, as Hasegawa *et al.* (Hasegawa *et al.*, 1986) noted that the extent of degeneration varied according to the degree of damage incurred and the time lapse present from injury. Because of the probability of bleeding and fibrosis at the cribiform plate, treatment should be directed at newer (yet to be developed) surgical attempts at lysis of this scar tissue.

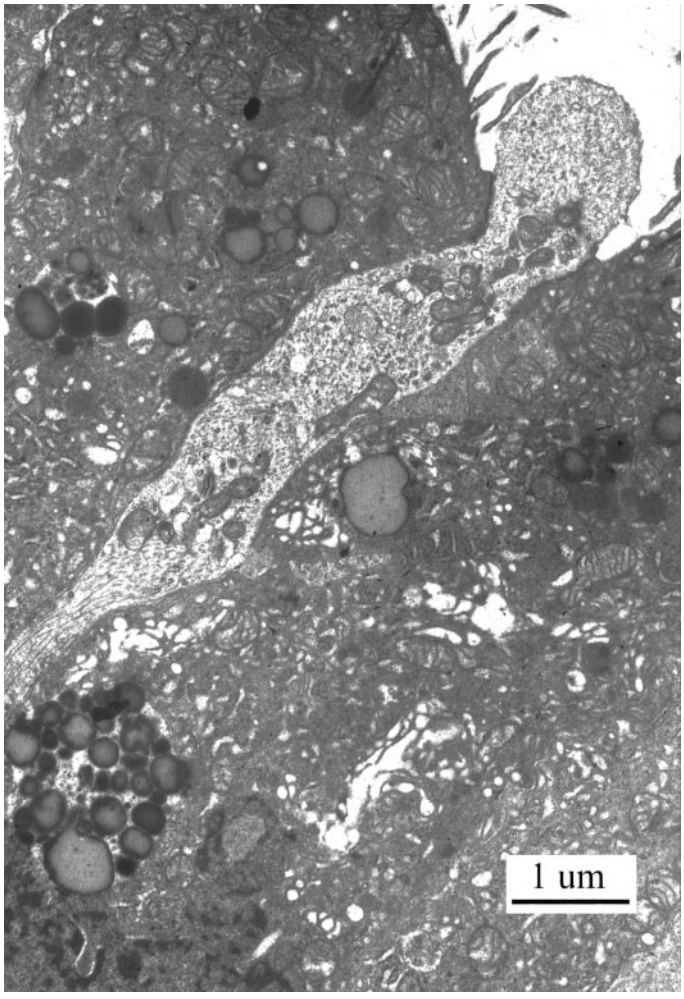

Figure 2 High power transmission electron micrograph (TEM) of olfactory epithelium from a patient with post-traumatic anosmia. Note the absence of olfactory cilia or basal bodies in the projecting olfactory vesicle.

Post-viral olfactory dysfunction (PVOD) is diagnosed when smell loss follows an upper respiratory infection. These patients tend to be older, with a 2:1 female preponderance (the reason for this is unknown) (Jafek *et al.*, 1990b). Hyposmia is more common and dysosmia may be seen in up to two-thirds of patients. Biopsy studies suggest a direct insult to the olfactory neuroepithelium with a 'patchy' regeneration accounting for the high incidence of dysosmia (Figure 3). Alternatively, patchy degeneration is also possible (Jafek *et al.*, 1990b). Looking at the residual olfactory epithelium, we found that in patients with anosmia the olfactory epithelium was markedly disorganized. Very few, if any, receptors were seen. Those that were present usually had dendrites that did not reach the epithelial surface and appeared somewhat shrunken. In hyposmic patients the individual receptors appeared much more normal, but reduced in numbers and distributed in 'patches'.

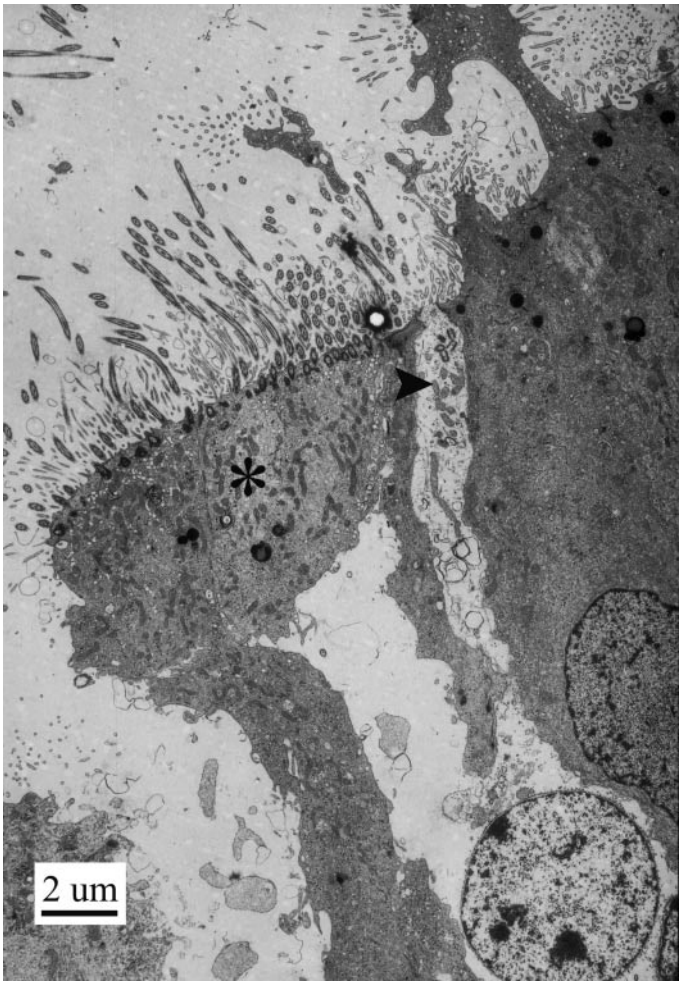

Figure 3 High power transmission electron micrograph (TEM) of olfactory epithelium from a patient with post-viral anosmia. A dendritic process or an olfactory receptor cell (arrowhead) is seen immedicately adjacent to a ciliated respiratory cell (asterisk), revealing a junction of respiratory epithelium (left) with the olfactory epithelium (right). See also discussion of the significance of these 'junctions' in the body of the paper.

On biopsy, frequent junctions of olfactory and respiratory epithelium are seen (Figure 3), suggesting an extremely patchy distribution of the olfactory epithelium ('checkerboard-like') interspersed with respiratory epithelium. The dendrites of the receptor cells contained large numbers of cytoplasmic inclusions reminiscent of myelin figures. Although the functional significance of the presence of these electron-dense bodies is unknown, their presence was remarkably consistent and limited to receptor cells. Microvillar cells were observed and appeared normal, as did the support cells, basal cells and underlying basement membrane. These findings were consistent with subsequent ones by Yamagishi *et al.* (Yamagishi *et al.*, 1994), who studied biopsies using immunohistochemical staining for neuron-specific enolase, S-100 protein, cytokeratin and proliferating cell nuclear antigen (PCNA). Although the recovery of olfactory function in patients with post-viral olfactory disorders was generally 'not very good', they found that overall a high proportion of Alinamin i.v. injection test-positive patient's recovered their sense of smell. Immunohistochemical study of the biopsy specimens revealed a decrease in the number of olfactory receptor cells and nerve bundles. In a few cases the olfactory neuroepithelium was replaced by metaplastic squamous epithelium. Sometimes different types of degeneration were found in the same specimen. No PCNA immunoreactivity was detected in the olfactory epithelium. They noted that recovery generally correlated with the initial degree of degeneration of the olfactory mucosa. Alinamin test-positive patients (generally the hyposmic patients) had more olfactory receptor cells. They concluded that olfactory mucosal biopsy and the Alinamin i.v. injection test were useful methods of determining the prognosis in post-URVI olfactory disorders (Yamagishi *et al.*, 1994). While the loss in PVOD does not fluctuate, gradual improvement in function over a period of years is often seen. Medical or surgical therapy has not generally been helpful.

Inflammatory nasal and sinus disease is one of the most common causes of olfactory loss (Seiden, 1997a,b). Obstruction of the olfactory cleft, or a 'conductive loss', is postulated to be etiologic, with a recurrent cycle of secretion stagnation, ciliary and epithelial damage and mucosal change. Histopathologically, the olfactory epithelium is initially normal in sinusitis (Jafek *et al.*, 1994; Seiden, 1997a,b). However, with recurrent infection and epithelial damage or concurrent viral infection, irreversible damage of the olfactory receptors with squamous metaplasia or fibrosis may result, making the olfactory dysfunction permanent (Jafek *et al.*, 1994; Seiden, 1997a,b). This condition is to be differentiated from steroid-dependent anosmia.

Steroid-dependent anosmia is a special type of sinonasal disease in which the nose contains a large number of polyps. It is differentiated from irreversible loss, as described above, by the 'burst of steroids test', as described by Jafek *et al.* (Jafek *et al.*, 1987). When this clinical test is positive, a burst of steroids (60 mg prednisone, decreasing by 5 mg/day, covered by prophylactic antibiotics) relieves the anosmia, temporarily, while the steroids are taken. Examination of the olfactory epithelium shows it to be entirely normal. Failure of the steroid test suggests irreversible extensive inflammatory disease or olfactory epithelial fibrosis, secondary to recurrent or chronic sinusitis or post-viral anosmia (Jafek *et al.*, 1987).

Olfactory biopsy has been helpful in a few other conditions. In congenital anosmia (e.g. Kallman's syndrome) we observed an absence or a severe decrease in the olfactory receptors, but those that were present appeared normal (Jafek *et al.*, 1990a). This agreed with Rawson *et al.* (Rawson *et al.*, 1995), who found that at least some olfactory neurons were functionally mature, by morphology and by calcium imaging, suggesting that complete development of the olfactory bulbs is not required for differentiation of mature

olfactory receptors. Others have observed that the neurons that were present lacked cilia (i.e. were morphologically immature), that the fila olfactoria had fewer than the normal number of axons and a large proportion of them were apparently undergoing electron-lucent degeneration and that neuromatous collections of axons were seen superficial to the basement membrane in the epithelium (Yousem *et al*., 1996). They characterized these changes as similar to those observed in the mucosa of experimentally bulbectomized rodents. This would be consistent with the absence of olfactory bulbs seen on MRI.

Neurodegenerative disorders (e.g. Wernicke's encephalopathy, Parkinson's disease, AIDS and dementia) have a variety of non-specific changes in the olfactory epithelium (Jafek *et al*., 1992). In Alzheimer's disease there is severe epithelial disruption, increased numbers of large mitochondria in the support cells with electron-dense particles and crystals on the overlying surface on the epithelium that stain metachromatically with toluidine blue (Moran *et al*., 1992). Radiographic microanalysis of these crystals indicates a high concentration of silicone in the area of the crystal deposit, while the adjacent respiratory epithelium exhibits no similar deposit of these crystals. Neurofilament antibodies and abnormal neuronal structures are also seen in the olfactory epithelium of patients with Alzheimer's disease, although Trojanowski *et al*. (Trojanowski *et al*., 1991) observed that dystrophic olfactory neurites occur very frequently in neurologically normal adults. The relevance of these neuritic changes to aging or specific disease processes remains speculative, however (Trojanowski *et al*., 1991). Aging produces a decrease in olfactory acuity, probably on the basis of a decrease in absolute numbers of receptors (Weiffenbach, 1984).

Toxic causes of olfactory dysfunction probably also produce decreased numbers of receptors, probably with intervening fibrosis (Hastings and Miller, 1997). Hepatic dysfunction, endocrine dysfunction, renal dysfunction and metabolic and nutritional deficiencies, along with a variety of pharmacological etiologies, are also described as causing olfactory loss. The exact nature of the epithelial changes has not yet been elucidated (Jafek *et al*., 1997).

Finally, as many as 29% of patients presenting with chemosensory complaints have no identifiable taste or smell loss, using standard tests (Doty *et al*., 1991). Here, biopsy would not be indicated, but should show normal epithelium.

Outlook

Olfactory marker protein (OMP) has been shown to be a robust marker of olfactory receptor neurons (Margolis, 1972). This will continue to be helpful in evaluating olfactory biopsies. Immunoelectron microscopy may aid in evaluating the morphology of OMP-immunoreactive neurons in various conditions (Johnson *et al*., 1989). Immunocytochemical studies using antisera against nerve growth factors will help to define the importance of nerve growth factor in various regenerative or developmental conditions. Additional studies of molecular changes in the components of the receptor cell membrane and receptors, amplified by PCR, should also help us understand the olfactory transduction mechanism, along with dysfunctional states (McClintock, 2000). Additional studies of ultrastructure, immunocytochemistry, molecular biology and physiology will continue to provide essential complementary information in elucidating olfactory pathophysiology.

In conclusion, olfactory biopsy of the olfactory epithelium has been shown to be a safe, reliable method to study both normal and pathological olfactory states over the past more than 20 years. Complications related to biopsy have been infrequent and minimal. Pre-biopsy olfactory acuity has been maintained. Additional studies of ultrastructure, complemented by immunocytochemical and molecular function, will continue to provide increased understanding of olfactory function and dysfunction, pointing the way to new therapeutic strategies.

References

Amoore, J.E. (1986) *Effects of chemical exposure on olfaction in humans*. In Barrow, C.S. (ed.), Toxicology of the Nasal Passages. Hemisphere Publishing, Washington, DC.

Cain, W.S., Gent, J.F., Goodspeed, R.B. and **Leonard, G.** (1988) *Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center*. Laryngoscope, 98, 83–98.

Doty, R.L., Shaman, P. and **Dann, M.** (1984) *Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function*. Physiol. Behav., 32, 489–502.

Doty R.L., Bartoshuk L. and **Snow, J.B.** (1991) *Causes of olfactory and gustatory disorders*. In Getchell, T.V., Doty, R.L., Bartoshuk, L.M. and Snow, J.B. (eds), Smell and Taste in Health and Disease. Raven Press, New York, NY, pp. 449–462.

Doty, R.L., Yousem, D.M., Pham, L.T., Kreshak, A.A., Geckle, R. and **Lee, W.W.** (1997) *Olfactory dysfunction in patients with head trauma*. Arch. Neurol., 54, 1131–1140.

Hasegawa, S., Yamagishi, M. and **Nakano, Y.** (1986) *Microscopic studies of human olfactory epithelia following traumatic anosmia*. Arch. Otolaryngol., 243, 112–116.

Hastings, L. and **Miller, M.L.** (1997) *Olfactory loss secondary to toxic exposure*. In Seiden, A.M. (ed.), Taste and Smell Disorders. Thieme, New York, NY, pp. 88–106.

Hill, D.P. and **Jafek, B.W.** (1989) *Initial otolaryngologic assessment of patients with taste and smell disorders*. Ear Nose Throat J., 68, 362–370.

Jafek, B.W. (1983) *Ultrastructure of human nasal mucosa*. Laryngoscope, 93, 1576–1599.

Jafek, B.W, Moran, D.T., Eller, P.M. and **Rowley, J.C.** (1987) *Steroid dependent anosmia*. Arch. Otol., 113, 547–549.

Jafek, B.W., Eller, P.M., Esses, B.A. and **Moran, D.T.** (1989) *Post-traumatic anosmia: ultrastructural correlates*. Arch. Neurol., 46, 300–304.

Jafek, B.W., Gordon, A.S.D., Moran, D.T. and **Eller, P.M.** (1990a) *Congenital anosmia*. Ear Nose Throat J., 69, 331–337.

Jafek, B.W., Hartman, D., Eller, P.M., Johnson, E.W., Strahan, R.C. and **Moran, D.T.** (1990b) *Post-viral olfactory dysfunction*. Am. J. Rhinol., 4, 91–100.

Jafek, B.W., Eller, P.M., Johnson, E.W., Chapman, M.M. and **Filley, C.M.** (1992) *Ultrastructural changes in the olfactory epithelium in Alzheimer's disease*. Am. J. Rhinol., 6, 219–225.

Jafek, B.W., Murrow, B. and **Johnson, E.W.** (1994) *Olfaction and endoscopic sinus surgery*. Ear Nose Throat J., 73, 548–552.

Jafek, B.W., Johnson, E.W., Eller, P. and **Murrow, B.** (1997) *Olfactory mucosal biopsy and related histology*. In Seiden, A.M. (ed.), Taste and Smell Disorders. Thieme, New York, NY, pp. 107–127.

Jafek B.W., Linschoten M. and **Murrow, B.** (2000) *Evaluation and treatment of anosmia*. Curr. Opin. Otolaryngol. Head Neck Surg., 8, 63–67.

Johnson, E.W., Eller, P.M. and **Jafek, B.W.** (1989) *Immunocytochemical investigation of olfactory neurons and synapses at the light and electron microscopic levels*. Chem. Senses, 14, 715.

Knecht, M., Kuhnau, D., Huttenbrink, K.B., Witt, M. and **Hummel, T.** (2001) *Frequency and localization of the putative vomeronasal organ in humans in relation to age and gender*. Laryngoscope, 111, 448–452.

Lanza, D.C., Moran, D.T., Doty, R.L., Trojanowski, J.Q., Lee, J.H., Rowley, J.C., Crawford, D. and **Kennedy, D.W.** (1993) *Endoscopic human olfactory biopsy technique: a preliminary report*. Laryngoscope, 103, 815–819.

Lanza, D.C., Deems, D.A., Doty, R.L., Moran, D., Crawford, D., Rowley, J.C., Sajjadian, A. and **Kennedy, D.W.** (1994) *The effect of human olfactory biopsy on olfaction: a preliminary report*. Laryngoscope, 104, 837–840.

Linschoten, M.R., Harvey, L.O. Jr, Eller, P.M. and **Jafek B.W.** (2001) *Fast and accurate measurement of taste and smell thresholds using a maximum-likelihood adaptive staircase procedure*. Percept. Psychophys., 63, 1330–1347.

Lovell, M.A., Jafek, B.W., Moran, D.T. and **Rowley, J.C.** (1982) *Biopsy of human olfactory mucosa: an instrument and a technique*. Arch. Otol., 108, 247–249.

Margolis, F.L. (1972) *A brain protein unique to the olfactory bulb*. Proc. Natl Acad. Sci. USA, 69, 1221–1224.

McClintock, T.S. (2000) *Molecular biology of olfaction*. In Finger, T., Silver, W.L. and Restrepo, D. (eds), The Neurobiology of Taste and Smell, 2nd Edn. Wiley-Liss, New York, NY, pp. 179–200.

Meredith, M. (2001) *Human vomeronasal organ function: a critical review of best and worst cases*. Chem. Senses, 26, 433–445.

Monti-Bloch, L., Jennings-White, C. and **Berliner, D.L.** (1998) *The human vomeronasal system. A review*. Ann. N.Y. Acad. Sci., 855, 373–389.

Moran, D.T., Jafek, B.W. and **Rowley, J.C.** (1991) *The vomeronasal (Jacobson's) organ in man: ultrastructure and frequency of occurrence*. J. Steroid Biochem. Mol. Biol., 39, 545–552.

Moran, D.T., Jafek, B.W., Eller, P.M. and **Rowley, J.C.** (1992) *Ultrastructural histopathology of human olfactory dysfunction*. Microsc. Res. Tech., 23, 103–110.

Murrow, B.M., Restrepo, D. and **Jafek B.W.** (2000) *A novel isolation system for human olfactory receptor cells*. Chem. Senses, 25, 5.

Rawson, N.E., Brand, J.G., Cowart, B.J., Lowry, L.D., Pribitkin, E.A., Rao, V.M. and **Restrepo, D.** (1995) *Functionally mature olfactory neurons from two anosmic patients with Kallmann syndrome*. Brain Res., 681, 58–64.

Schiffman, S.S. (1983) *Taste and smell in disease*. N. Engl. J. Med., 308, 1337–1343.

Seiden, A. (ed.) (1997a) Smell and Taste Disorders. Thieme, New York, NY.

Seiden, A. (1997b) *Olfactoy loss secondary to nasal and sinus pathology*. In Seiden, A. (ed.), Smell and Taste Disorders. Thieme, New York, NY, pp. 52–71.

Trojanowski, J.Q., Newman, P.D., Hill, W.D. and **Lee, V.M.** (1991) *Human olfactory epithelium in normal aging, Alzheimer's disease, and other neurodegenerative disorders*. J. Comp. Neurol., 310, 365–376.

Truit, C.L. and **Kelly, W.M.** (1993) *The olfactory system*. Neuroimaging Clin. N. Am., 3, 47–70.

Weiffenbach, J.M. (1984) *Taste and smell perception in aging*. Gerodontology, 3, 131–136.

Yamagishi, M. and **Nakano, Y.** (1992) *A re-evaluation of the classification of olfactory epithelia in patients with olfactory disorders*. Eur. Arch. Otorhinolaryngol., 249, 393–399.

Yamagishi, M., Fujiwara, M. and **Nakamura, H.** (1994) *Olfactory mucosal findings and clinical course in patients with olfactory disorders following upper respiratory viral infection*. Rhinology, 32 113–118.

Yousem, D.M., Geckle, R.J., Bilker, W., McKeown, D.A. and **Doty, R.L.** (1996) *MR evaluation of patients with congenital hyposmia or anosmia*. Am. J. Roentgenol., 166, 439–443.

Accepted May 25, 2002