with minimum trauma, and the opening persists as long as the graft is in place (*16*). In this transplantation system, in which the brain is left as undisturbed as possible, vessels appear to emerge from the brain parenchyma to supply the graft. This vascular response suggests that some angiotropic factor produced by the graft induces changes in the host endothelium. The vasculature in the graft of autonomic neural tissue is highly permeable to blood-borne protein, just as it is in situ. We previously found that intravascular administration of the catecholamine analog 5-hydroxydopamine (molecular weight 205), which normally cannot cross the blood-brain barrier, labeled noradrenergic boutons and storage sites in the graft (*12*). Direct anastomoses were formed between the vessels of the graft and adjacent tissue; India ink, infused into the aorta, filled vessels from the choroid plexus, medulla, and cerebellum that were directly confluent with graft vessels. It is not known whether the graft vessels are replaced by those growing from the surrounding central nervous system (*17*). Electron microscopy reveals fenestrated vessels in the SCG transplant that resemble those of the SCG in situ (*12*). This finding and the fact that SCG grafts are vascularized within 18 to 24 hours (*12, 17*) would argue against the inference proposed for skin and iris implants (*2*): that the original capillary bed of the graft dies and is completely replaced by vessels from the host. Rather, some of the blood vessels that persist within the graft may be derived from original, intrinsic vessels, inasmuch as they are readily permeable to protein and amine.

Jacobs *et al.* (*18*) recently suggested that the lack of a barrier system in ganglion tissue exposes it to environmental toxins that are excluded from the central nervous system. We have determined that the difference between peripheral and central neurovasculature is apparently maintained indefinitely in this transplantation system. Transplants of autonomic neural tissue can provide a permanent biological portal whereby blood-borne substances, including biologically active peptides, hormones, and immunoglobulins, can be delivered directly to adjacent brain areas.

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References and Notes

1. R. D. Lund and S. D. Hauschka, *Science* **193**, 582 (1976); A. Bjorklund and U. Stenevi, *Brain Res.* **177**, 555 (1979); M. J. Perlow *et al.*, *Science* **204**, 643 (1979); D. Gash, J. R. Sladek, Jr., C. D. Sladek, *ibid.* **210**, 1367 (1980).
2. N.-A. Svendgaard, A. Bjorklund, J.-E. Hardebo, J.-E. Stenevi, U. Stenevi, *Nature (London)* **225**, 334 (1975).
3. J. M. Rosenstein and M. W. Brightman, *ibid.* **275**, 83 (1978).
4. ———, *Neurocytology* **8**, 359 (1979).
5. M. Yamamoto, V. Chan-Palay, H. Steinbusch, S. Palay, *Anat. Embryol.* **159**, 1 (1980); A. McCrae-Deguerce, M. Didier, J. Pujol, *Neurosci. Lett.* **24**, 251 (1982).
6. M. I. Phillips, K. Hermsmyer, S. Landas, G. Hadley, J. Gerndt, *Life Sci.* **27**, 2583 (1980).
7. J. M. Rosenstein and M. W. Brightman, *Peptides* **1**, 221 (1980); *J. Neurocytol.* **10**, 387 (1981).
8. J. M. Jacobs, *J. Neurocytol.* **6**, 607 (1977).
9. R. C. Graham and M. J. Karnovsky, *J. Histochem. Cytochem.* **14**, 291 (1966).
10. M. W. Brightman, M. Hori, S. Rappaport, T. S. Reese, E. Westergaard, *J. Comp. Neurol.* **152**, 317 (1973).
11. I. Klatzo, *J. Neuropathol. Exp. Neurol.* **26**, 1 (1967).
12. J. M. Rosenstein and M. W. Brightman, in *Neural Transplants: Development and Function*, J. Sladek and D. Gash, Eds. (Plenum, New York, in press).
13. E. Westergaard, B. van Deurs, H. Bronsted, *Acta Neuropathol.* **37**, 141 (1977).
14. J. T. Povlishock, D. P. Becker, H. G. Sullivan, J. D. Miller, *Brain Res.* **153**, 223 (1978).
15. D. Groothius *et al.*, *J. Neuropathol. Exp. Neurol.* **41**, 164 (1982).
16. Hypertensive and hyperosmotic methods result in an opening that is random, disseminated, and reversible. Opening by cerebral trauma is transient and destructive. Although some cerebral tumors have vessels that remain permeable indefinitely, it is difficult to confine them to a selected region and size.
17. S. Tsubaki and M. Brightman, unpublished observations. Studies are being conducted with gamma-glutamyl transpeptidase, which is present in central vessels but not SCG vessels. In addition, autoradiographic labeling studies of host and donor vascular cells have been initiated (J. Krum and J. Rosenstein, in preparation).
18. J. M. Jacobs, R. M. MacFarlane, J. B. Cavanough, *J. Neurol. Sci.* **29**, 95 (1976).
19. Supported in part by NIH grant NS-17468 to J.M.R.
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Otosclerotic Lesions in the Inbred LP/J Mouse

Abstract. *Inbred LP/J mice develop abnormal bony lesions that are grossly and histologically similar to the lesions of human otosclerosis. This is the first known occurrence of spontaneous otosclerosis-like lesions in an animal. As in the human disease, these lesions impair audition by immobilizing the ossicles of the middle ear. The LP/J mouse may be an animal model for this common human disease.*

Otosclerosis, an inherited disease in which foci of abnormal bone develop in the middle and inner ear, affects 14 to 15 million people in the United States (*1*). Ten percent of affected individuals experience progressive hearing loss because of gradual immobilization of the stapes by abnormal bone. In addition, when otosclerotic bone involves the endosteum of the otic capsule, progressive damage to the inner ear may result in sensorineural hearing loss. Although highly successful surgical procedures have been devised to correct the ossicular fixation, there is no treatment for the cochlear damage. This disease has been thought to occur spontaneously only in humans, and attempts at experimental induction in animals have not been entirely successful. Therefore, knowledge of its pathophysiology is poorly understood. In this report we describe spontaneous development of otosclerosis-like foci in an animal (*2*).

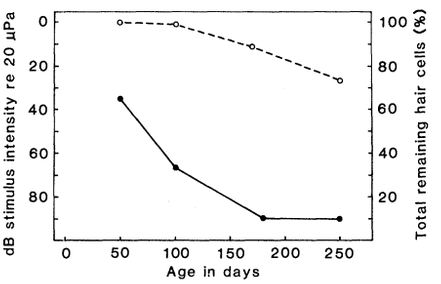

Fig. 1. Mean hair cell loss in the entire cochlea (○) and mean threshold of the auditory evoked response (●) in LP/J mice of various ages. Auditory acuity diminishes rapidly with age while the hair cell population changes little. These data were derived from cytocochleograms and electrocochleograms.

The inbred LP/J mouse has a progressive loss of auditory function (*3*); however, examination reveals that the hair cells of the cochlea remain remarkably intact when the animal has little or no auditory function (Fig. 1) (*4*). The scanning electron microscope confirms the normal appearance of the hair cells in animals with marked shifts in auditory threshold. While examining the middle ear structures of these mice, we observed abnormal bony structures on the ossicles (Fig. 2). Since these lesions are grossly similar to the lesions of human otosclerosis, we performed a histological and ultrastructural study of these ossicles.

Adult LP/J mice between 100 and 250 days of age were reared in a colony in which ambient noise was less than 40 dB over the range of their hearing. These animals were first- to third-generation descendants of parental stock obtained from the Jackson Laboratory at Bar Harbor, Maine. They were given unlimited water and Simonson mouse food. Each animal was deeply anesthetized intraperitoneally with pentobarbital and its bulla was flooded with 2 percent paraformaldehyde and 2 percent glutaraldehyde in 0.08*M* cacodylate buffer (*p*H 7.4

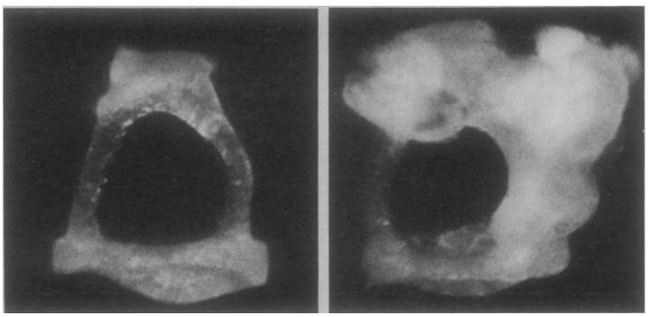

Fig. 2. Normal stapes from a CBA/J mouse (left) and stapes with abnormal bone deposits from an LP/J mouse (right) (×66).

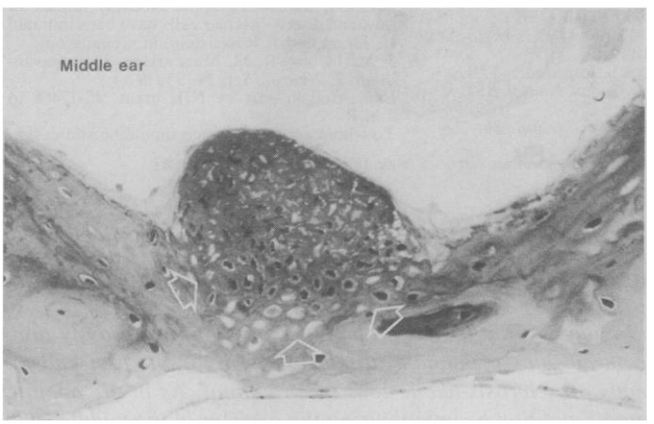

Fig. 3. Section of an abnormal focus of bone adjacent to the stapes of a 150-day-old LP/J mouse, showing a pattern similar to that seen in human otosclerosis. New bone is being deposited and the underlying normal bone is eroding (arrows). The abnormal bone contains more cells than the surrounding bone. Stain, basic fuchsin and methylene blue (×280).

at 4°C). After 4 hours of fixation, selected specimens were decalcified in 0.1*M* EDTA and 5 percent glutaraldehyde in 0.08*M* cacodylate buffer and postfixed in 2 percent OsO_4 with buffer for 2 hours. The washed tissue was then dehydrated in acetone, embedded in Epon-Araldite, and sectioned on an ultramicrotome for light and electron microscopy. Thin sections were placed onto 200-mesh copper grids and stained with uranyl acetate and lead citrate before being viewed under a Zeiss 109 transmission electron microscope.

The chalky white deposits seen on the ossicles and otic capsule were histologically indistinguishable from human otosclerotic tissue. The bony lesions appeared to distort the normal ossicular contours and did not seem to be merely new bone deposits (Fig. 3). Most lesions had a greater ratio of cells to bone matrix than the surrounding normal bone of the ossicles and otic capsule. Some areas appeared to be cartilaginous and some to be areas of calcifying osteoid with calcospherites when viewed by electron microscope. The abnormal bone varied markedly from one location to another. Some areas seemed to be sclerotic, having dense bone and small lacunae with osteoclasts. Other areas appeared spongiotic, with large cellular areas showing poor mineralization. Still other areas appeared to have "blue mantles" around cellular spaces, corresponding to preotosclerotic human tissue (*5*).

The ultrastructural features were also similar to those of the human disease. The lesions showed evidence of both bone destruction and deposition. Active bone deposition probably best typifies active otosclerosis or, more accurately, otospongiosis. As with human spongiotic lesions (*5*, *6*), the actively growing lesions in the LP/J mouse were areas of osteoblastic activity where calcospherites were being deposited on poorly organized collagen fibers surrounded by amorphous ground substance.

We examined 22 ears from 11 animals and observed the lesions in 9 ears (41 percent). The lesions were seen in males and females, were frequently bilateral, and were seen most commonly in the ossicles but were also present in other locations in the otic capsule. No lesions were observed outside the temporal bone. Mice of other genotypes (CBA, AUSs, SJL, C57BL/6, AKR, and C57BR/cd) which were simultaneously raised under identical conditions in our colony did not develop the bone lesions seen in the LP/J mice.

Other investigators have induced otosclerosis-like lesions with venous stasis (*7*), Vigantol poisoning (*8*), electrocoagulation (*9*), sodium morrhuate, and ultrasound (*10*). However, these lesions did not result in hearing loss and were not hereditary in origin. Mendoza *et al.* (*10*) produced bony lesions in the endochondral bone of the otic capsule with 5280 to 8330 rads of cobalt-60 irradiation. These lesions looked otosclerotic under the light microscope, but, since they were not genetically transferred and did not involve the ossicles or cause hearing loss, they are not a convincing model for human otosclerosis. Yoo and colleagues (*11*) produced otosclerosis-like bone lesions in rat otic capsules by inducing type II collagen immunity, but did not confirm this histologically.

The inbred LP/J mouse spontaneously develops otosclerosis-like lesions of the ossicles of the middle ear and the bone of the otic capsule, causing immobility of the ossicles and conductive auditory impairment. The similarity of this apparently inherited bone disease to human otosclerosis suggests that the LP/J mouse may be useful as an animal model for the human disease.

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References and Notes

1. G. A. Gates, *Ann. Otol. Rhinol. Laryngol.* **91** (6-3), Suppl. 100 (1982).
2. Otosclerosis-like lesions in the dog were described by G. Alexander [*Arch. Ohrenheilkd.* **78**, 3 (1909)], but no anatomical or histological evidence was given. No such lesions have been observed by other investigators.
3. K. R. Henry, *J. Gerontol.* **37**, 275 (1982).
4. R. A. Chole and K. R. Henry, *Audiology*, in press.
5. D. J. Lim and W. H. Saunders, *Ann. Otol. Rhinol. Laryngol.* **86**, 525 (1977).
6. L. G. Chevance, M. B. Jorgensen, P. Bretalu, J. Causse, *Acta Otolaryngol.* **67**, 563 (1969).
7. K. Wittmaack, *ibid.* **14**, 228 (1930).
8. W. Krainz, *Monatsschr. Ohrenheilkd. Laryngo-Rhinol.* **70**, 6 (1936).
9. R. J. Bellucci and D. Wolft, *Arch. Otolaryngol.* **71**, 224 (1960).
10. D. Mendoza, M. R. deStefani, F. Leborgne, *Acta Otolaryngol.* **67**, 9 (1969).
11. T. J. Yoo *et al.*, *Science* **217**, 1153 (1982).
12. Supported by PHS grants 1 K07 NS00396-05 and 1 R01 NS17208-2.

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