newly inserted receptors share other properties of extrajunctional receptors.

The finding of a subpopulation of rapidly degraded ACh receptors in the junctional region may shed light on certain puzzling observations. First, the observation that neuromuscular transmission recovers more quickly than expected after blockade of ACh receptors with the irreversible agent α-BuTx (*3, 14*) might be explained by the more rapid rate of junctional receptor synthesis predicted by our results; a proportion of the blocked ACh receptors would thus be quickly replaced by newly synthesized receptors. Second, the rapid degradation of a subpopulation of receptors may help to explain discrepancies in previous estimates of junctional ACh receptor turnover rates; estimates of half-lives of junctional ACh receptors range from 6 to 13 days. Measurements of receptor loss begun soon after labeling and continued for only a short period would give an apparently faster rate of junctional ACh receptor turnover and a correspondingly shorter half-life than measurements begun later after labeling, or for longer periods, when the subpopulation of rapidly degraded receptors would have less influence on the overall receptor degradation rate. For example, in an earlier study we had examined degradation occurring for a relatively short time and found a half-life of 5.6 days for junctional ACh receptors (*6*) as compared with the half-life of 12.4 days obtained in the present study in which turnover was examined later after labeling and for a longer time period.

The coexistence of rapidly and slowly degraded ACh receptors at the end plate can be explained by two hypotheses. First, it is possible that ACh receptors are initially inserted into the postsynaptic membrane in a form that is relatively unstable and that they are subsequently stabilized by some influence of the motor nerve. Alternatively, there may be two or more independent pools of ACh receptors with different intrinsic turnover rates. Earlier observations that the slow degradation rate of preexisting junctional ACh receptors can be increased after denervation indicate that junctional ACh receptors can be destabilized by removing the influence of the motor nerve (*7, 10*). Furthermore, ACh receptors that are inserted into the junctional region after a period of denervation have a turnover rate that is very rapid and is similar to that of extrajunctional ACh receptors (*15*). Taken together, these observations favor the nerve stabilization hypothesis. Reiness and Weinberg (*16*) have shown that receptors in new ectopic synapses have a rapid rate of turnover and may correspond to the rapidly degraded receptors identified in this study at the intact, mature neuromuscular junction. How the nerve might influence the stability of ACh receptors at the neuromuscular junction remains to be determined. It could act directly by altering the ACh receptors or their immediate environment or indirectly by inducing a change in the postsynaptic membrane or underlying cytoskeleton of the muscle cell.

ELIS F. STANLEY
DANIEL B. DRACHMAN

Department of Neurology,
Johns Hopkins Unversity
School of Medicine,
Baltimore, Maryland 21205

References and Notes

1. D. K. Berg and Z. W. Hall, *Science* **184**, 473 (1974).
2. C. C. Chang and M. C. Huang, *Nature (London)* **253**, 634 (1975).
3. D. K. Berg and Z. W. Hall, *J. Physiol. (London)* **252**, 771 (1975).
4. D. M. Fambrough, *Physiol. Rev.* **59**, 165 (1979).
5. D. C. Linden and D. M. Fambrough, *Neuroscience* **4**, 527 (1979).
6. E. F. Stanley and D. B. Drachman, *Science* **200**, 1285 (1978).
7. T. A. Levitt and M. M. Salpeter, *Nature (London)* **291**, 239 (1981).
8. J. P. Merlie, S. Heinemann, J. M. Lindstrom, *J. Biol. Chem.* **254**, 6320 (1979).
9. P. M. Devreotes and D. M. Fambrough, *J. Cell Biol.* **65**, 335 (1975).
10. E. F. Stanley and D. B. Drachman, *Exp. Neurol.* **73**, 390 (1981).
11. The location of ^{125}I-α-BuTx binding sites in the junctional and perijunctional region was determined by light autoradiography. At 1, 6, and 15 days after ^{125}I-α-BuTx labeling in vivo, diaphragms were removed, washed, fixed in 2 percent glutaraldehyde, washed in phosphate buffer, and teased into single fibers on gelatin-coated slides in a drop of 5 percent albumen [S. Burden, *Dev. Biol.* **57**, 317 (1977)]. The slides were dipped in Kodak emulsion NBT2, allowed to dry, and left to expose for 24 hours at room temperature. Grains in the junctional and perijunctional regions were counted as follows. The muscle fiber was positioned under the microscope at ×100 so that a 50-μm square grid in the eyepiece was located over the densest accumulation of grains. This was defined as the junction-containing strip of the muscle fiber. The 50-μm strips on either side of this region were defined as the perijunctional strips. Background binding was determined both from the slide in a region away from the muscle fibers and from single, teased fibers from an extrajunctional segment of the diaphragm. Grain counts on the extrajunctional muscle fibers were not significantly higher than counts on an equivalent area of the slide. All grains on the muscle fiber and within 5 μm on either side of the fiber were included in the counts, and background counts were subtracted. One day after labeling a mean ± standard deviation of 247 ± 95 grains (N = 23 fibers) were counted in the 50-μm junctional strip, and a total of 13.0 ± 11.3 grains were counted in the two 50-μm perijunctional strips.
12. The perijunctional strips contained only 2.5 percent as many grains as were present in the junctional strip (N = 23 fibers). The percentage in the perijunctional region did not decline at 6 days (4 percent, N = 16), nor 15 days (3.5 percent, N = 6) after labeling. Thus, during the first few days after labeling, when most of the rapid rate of loss of radioactivity occurred, there was no decrease in the proportion of perijunctionally bound ^{125}I-α-BuTx. Furthermore, when diaphragms were labeled 6 days after existing receptors were blocked with unlabeled α-BuTx, there was no greater percentage of grains in the perijunctional region (2. 6 percent, N = 15).
13. H. C. Fertuck and M. M. Salpeter *J. Cell Biol.* **69**, 144 (1976); N. Robbins, A. Olek, S. S. Kelly, P. Takach, M. Christopher, *Proc. R. Soc. London Ser. B* **209**, 555 (1980); S. W. Kuffler and D. Yoshikami, *J. Physiol. (London)* **244**, 703 (1975).
14. C. C. Chang, S.-T. Chuang, M. C. Huang. *J. Physiol. (London)* **250**, 161 (1975); H. C. Fertuck, W. Woodward, M. M. Salpeter, *J. Cell Biol.* **66**, 209 (1975); A. Pestronk and D. B. Drachman, *Science* **199**, 1223 (1978).
15. R. H. Loring and M. M. Salpeter, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2293 (1980); C. B. Weinberg, C. G. Reiness, Z. W. Hall, *J. Cell Biol.* **88**, 215 (1981).
16. C. G. Reiness and C. B. Weinberg, *Dev. Biol.* **84**, 247 (1981).
17. Supported by grants 5 P01 NS10920 and 5 R01 HD04817 from the National Institutes of Health. We thank A. Pestronk for helpful discussion, C. A. Padula for help with autoradiography, and C. Barlow-Salemi for preparation of the manuscript.

24 March 1983; revised 6 June 1983

Intracellular Recordings from Cochlear Inner Hair Cells: Effects of Stimulation of the Crossed Olivocochlear Efferents

Abstract. *Intracellular recordings were obtained from inner hair cells located in the lower basal turn of the guinea pig cochlea. At low sound pressure levels the inner hair cells were highly frequency selective, producing receptor potentials only in response to sound frequencies between about 16 and 24 kilohertz. Electrical stimulation of efferent nerves in the crossed olivocochlear bundle markedly reduced these receptor potentials while causing little change in the resting membrane potential. At high sound levels, where cells responded to an increasingly wider range of sound frequencies, stimulation was less effective in reducing receptor potentials. Since the crossed olivocochlear bundle primarily innervates outer hair cells, these results support an outer hair cell contribution to the most sensitive response region of inner hair cells.*

Octavo-lateralis sensory systems are characterized by an abundant efferent, as well as afferent, innervation (*1*). In the mammalian cochlea, the two types of sensory receptors, or hair cells, receive distinct patterns of innervation (Fig. 1A). Whereas inner hair cells (IHC's) receive the bulk (85 to 95 percent) of the afferent innervation (*2*), in the guinea pig, efferent endings on IHC's occur only rarely (*3*). Outer hair cells (OHC's) receive a large number of efferent endings, many of which are from axons of the crossed olivocochlear bundle (COCB), which originates in the brainstem in the medial portion of the superior olivary complex

and decussates at the level of the facial genu (*4*). Other efferent fibers to the OHC's may project from the superior olivary complex on the ipsilateral side (not shown in Fig. 1A). In addition, the COCB may contain some efferents that end on afferent dendrites beneath the inner hair cells (*5*).

Several studies have shown that electrical stimulation of the COCB reduces auditory afferent neural activity in response to sound (*6*). In studies of single afferent neurons, virtually every unit was affected by COCB stimulation (*7*). These results are perplexing when the cochlear innervation pattern is considered, since stimulation of axons that end primarily on OHC's affects the responses of neurons that transmit information from IHC's. Explanations for this paradox include the possibility of COCB endings on afferent neurons or the possible spread of stimulating current to the uncrossed olivocochlear bundle (UOCB), which may end in efferent synapses on afferent axons (Fig. 1A). If these explanations are correct, COCB stimulation should not alter the responses from IHC's. We tested these hypotheses by making intracellular recordings from IHC's during COCB stimulation.

Most of the experimental procedures used with anesthetized guinea pigs have been described (*8*). In our experiments, the IHC recording site is limited to the lower basal turn, an area most responsive to high frequencies. The physiological condition of this portion of the cochlea was verified by recording, from the round window, the compound action potential of the auditory nerve in response to high-frequency (16 to 22 kHz) tone bursts with a rise time of 1 msec. Only preparations in which action potential thresholds were unchanged by surgical procedures are considered here. The IHC's were identified by electrophysiological and histological criteria (*9*). For electrical stimulation, a bipolar electrode was implanted at the COCB (*10*). The middle ear muscles were sectioned, and the animals were paralyzed with curare to reduce movement artifacts.

Figure 1B shows an intracellular recording from one representative IHC. The sound stimuli were repeated tone bursts at the characteristic frequency, which is the sound frequency that evokes the largest d-c response at low sound pressure levels (SPL's). The d-c receptor potential is a depolarization of the membrane lasting for the duration of the sound stimulus. During a portion of the recording, the COCB was electrically stimulated with a train of pulses. (i) The resting membrane potential (when no sound stimulus was present) was relatively unaltered during COCB stimulation (*11, 12*). The IHC's never hyperpolarized during COCB stimulation. (ii) The d-c receptor potential was substantially reduced during COCB stimulation.

Figure 1C shows intensity, or input-output, functions for the IHC d-c receptor potential for normal conditions and during COCB stimulation. At the characteristic frequency, the normal function showed an increase in receptor potential with increasing SPL until about 45 dB, above which the function saturated. Stimulating the COCB decreased the receptor potential, especially over the initial portion of the curve. At the lowest SPL used, the 2.75-mV receptor potential was eliminated by COCB stimulation. At high SPL's, COCB stimulation was less effective in decreasing the response.

As a measure of the effectiveness of efferent stimulation over the rising portion of the intensity function, we computed Δ (Fig. 1C), which is the amount of increase in SPL required to bring the response during COCB stimulation up to the level of the normal response. For 12 IHC's with characteristic frequencies between 16 and 22 kHz, Δ ranged from 9.5 to 20 dB. In addition to the d-c receptor potential, the intracellular response also consists of an a-c receptor potential at the frequency of the sound stimulus (*9*). For each IHC, values of Δ for the two receptor potentials were similar. The compound action potential evoked by high-frequency tone bursts was also measured during COCB stimulation. In each guinea pig studied, values of Δ for the action potential were usually similar to those for the d-c receptor potential.

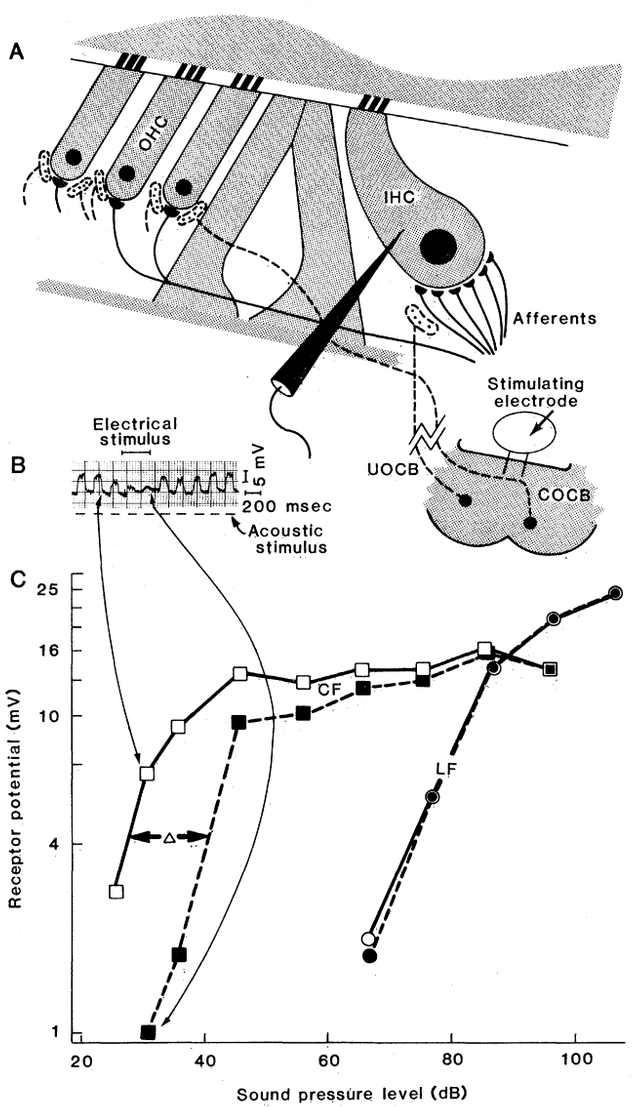

Fig. 1. (A) Simplified diagram of the hair cell innervation patterns in the mammalian cochlea. Afferent neurons are shown in solid lines, efferent neurons in dashed lines. An intracellular recording electrode is shown impaling an inner hair cell and an electrical stimulating electrode is shown in the brainstem. Abbreviations: *OHC*, outer hair cells; *IHC*, inner hair cell; *COCB*, crossed olivocochlear bundle; *UOCB*, uncrossed olivocochlear bundle. (B) Intracellular d-c receptor potentials in response to repeated tone bursts at the characteristic frequency (20 kHz) of the inner hair cell. The recording was made with the use of a direct-coupled amplifier. Electrical stimuli were presented to the COCB during the indicated interval. (C) Direct-current receptor potential intensity functions at the characteristic frequency (*CF*, 20 kHz) and a lower frequency (*LF*, 12.4 kHz) for normal conditions (solid lines) and during COCB stimulation (dashed lines). Values during COCB stimulation were obtained during the last 200 msec of the 400-msec trains of electrical stimuli. The sound pressure level is with respect to 20 μPa.

Figure 1C also shows an intensity function from the same IHC for a lower sound frequency. Higher SPL's were required to evoke responses and the function did not begin to saturate until higher receptor potential levels. The function was only slightly altered by COCB stimulation. For eight IHC's, low-frequency (6 to 12.5 kHz) intensity function Δ ranged from 0 to 2.4 dB.

We also investigated the effects of COCB stimulation on frequency tuning curves for the d-c receptor potential (Fig. 2). These curves graph the SPL required to evoke a 2-mV response against sound frequency. The normal frequency tuning curve has a sensitive response region consisting of a finely tuned "tip" with characteristic frequency of about 22 kHz, as well as a less sensitive response region consisting of a broad low-frequency "tail" (below 14 kHz). The dashed curve was obtained during a 20-second period of continuous COCB stimulation. The sensitivity of the tip was reversibly reduced, whereas the tail was unaltered. In seven tuning curves, tip sensitivity decreased from 5 to 17 dB.

The effects of COCB stimulation on IHC's are similar to many aspects of the effects of COCB stimulation on neural responses shown by earlier investigations (*7*). In the absence of sound stimulation, the IHC resting membrane potential, like the spontaneous activity of afferent neurons, is relatively unaltered by COCB stimulation. The most profound effect of COCB stimulation on IHC's and auditory neurons is to decrease responses evoked by sounds of low SPL with frequencies near the characteristic frequency. For auditory nerve fibers, Δ ranges from about 0 to 20 dB (*7*) and the Δ for IHC's falls within this range. We encountered no IHC's uninfluenced by COCB stimulation in our limited sample of undamaged cochleas.

Neural units tuned to middle and high frequencies have frequency tuning curves with well-defined tip and tail portions. The effect of COCB stimulation on these neural tuning curves is to decrease the sensitivity of the tip with less alteration of the tail (*13*), an effect also found for the IHC responses of this study, which are limited to hair cells tuned to high frequencies. Some auditory neurons tuned to low frequencies show sensitivity decreases below the characteristic frequency (*13*), but this effect may result from a different process, since the COCB innervation of OHC's in the apical, low-frequency portions of the cochlea is minimal (*14*).

We have shown that IHC responses

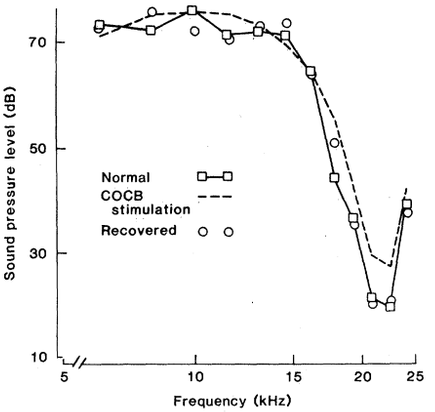

Fig. 2. Inner hair cell FTC's for the d-c receptor potential, with a 2-mV criterion response, during normal conditions (□—□), during a 20-second period of continuous COCB stimulation (– – –), and after COCB stimulation (○ ○).

can be altered by efferent COCB stimulation, in a pattern similar to the alteration of responses of afferent neurons, which transmit information from the IHC's. The effect of COCB stimulation on the afferent neural response thus occurs with the involvement of the IHC's. Hence, we rule out hypotheses that invoke COCB endings on afferent neurons, or spread of stimulation current to the UOCB, to explain the effects of COCB stimulation on afferent neural responses. Also, no electrophysiological evidence of direct COCB synapses onto IHC's was observed. This is consistent with cochlear anatomical evidence and contrasts with observations made on hair cells of the lateral line, frog sacculus, and turtle cochlea, where efferent fibers form axo-somatic synapses onto hair cells (*15, 16*). In these hair cells, the effect of efferent stimulation is to hyperpolarize the resting membrane potential by 5 to 25 mV (*16, 17*).

The alterations of IHC's by COCB stimulation may be explained by an OHC influence on the response of IHC's. An OHC contribution to the IHC response has been suggested, especially to explain substantial alterations of the most sensitive response region of afferent neurons observed after OHC's have been selectively destroyed with aminoglycoside antibiotics (*18, 19*). These earlier results have been criticized because of the possibility that the IHC's were directly affected by the drug treatment (*20*). Our study indicates that a hair cell interaction can take place in the undamaged cochlea, at least during COCB stimulation. No neural substrate for an OHC contribution to IHC responses has been found (*21*) and an electrical interaction has been proposed (*19*). Recent evidence, however, suggests that the interaction may be mechanical (*12, 22*), possibly via the outer hair cell stereocilia and the tectorial membrane.

M. Christian Brown*
Alfred L. Nuttall
Robert I. Masta
Kresge Hearing Research Institute, University of Michigan Medical School, Ann Arbor 48109

References and Notes

1. R. Klinke and N. Galley, *Physiol. Rev.* **54**, 316 (1974); S. Iurato, *Handbook of Sensory Physiology*, vol. 5, part 1, *Anatomy, Physiology (Ear)*, W. D. Keidel and W. D. Neff, Eds. (Springer-Verlag, Berlin, 1974), p. 261.
2. H. Spoendlin, *Acta Oto-Laryngol.* **67**, 239 (1969); D. Morrison, R. A. Schindler, J. Wersall, *ibid.* **79**, 11 (1975).
3. C. A. Smith, *Adv. Oto-Rhino-Laryngol.* **20**, 296 (1973); K. Saito, *J. Ultrastruct. Res.* **71**, 222 (1980).
4. G. L. Rasmussen, *J. Comp. Neurol.* **84**, 141 (1946); S. Iurato, *Exp. Cell Res.* **27**, 162 (1962); R. Kimura and J. Wersall, *Acta Oto-Laryngol.* **55**, 11 (1962).
5. W. B. Warr and J. J. Guinan, Jr., *Brain Res.* **173**, 152 (1979).
6. R. Galambos, *J. Neurophysiol.* **19**, 424 (1956); J. E. Desmedt, *J. Acoust. Soc. Am.* **34**, 1478 (1962); T. Konishi and J. Z. Slepian, *ibid.* **49**, 1762 (1971).
7. J. Fex, *Acta Physiol. Scand.* **55** (*Suppl.* 189), 1 (1962); M. L. Weiderhold, *J. Acoust. Soc. Am.* **48**, 966 (1970); _____ and N.Y.-S. Kiang, *ibid.*, p. 950; D. C. Teas, T. Konishi, D. W. Nielson, *ibid.* **51**, 1256 (1972); M. L. Gifford and J. J. Guinan, Jr., *ibid.* **74**, 115 (1983).
8. P. M. Sellick and I. J. Russell, in *Auditory Investigation: The Scientific and Technological Basis*, H. A. Beagley, Ed. (Oxford Univ. Press, Oxford, 1979), pp. 368; M. C. Brown, A. L. Nuttall, R. I. Masta, M. Lawrence, *Hearing Res.* **9**, 131 (1983).
9. I. J. Russell and P. M. Sellick, *J. Physiol. (London)* **284**, 261 (1978); D. A. Goodman, R. L. Smith, S. C. Chamberlain, *Hearing Res.* **7**, 161 (1982). In two cases, horseradish peroxidase was introduced by iontophoresis into cells electrophysiologically identified as IHC's and altered during COCB stimulation. In both cases, cells were identified histologically as IHC's.
10. A bipolar stainless steel wire electrode assembly [B. Oakley and R. Schafer, *Experimental Neurobiology: A Laboratory Manual* (Univ. of Michigan Press, Ann Arbor, 1978), p. 337] was implanted stereotaxically through the cerebellum and the fourth ventricle and into the COCB. Adjustments in position were made until the compound action potential evoked by a click was decreased by COCB stimulation the equivalent of decreasing the SPL between 8 and 20 dB. In some guinea pigs, correct placement of the COCB electrode was verified histologically by the Prussian blue reaction and counterstaining with Thionin. Electrical stimuli were led through a stimulus isolation unit and were pulses 0.3 msec in duration repeated at 400 Hz and presented either continuously or in trains 400 msec long.
11. The alterations in IHC resting membrane potential during COCB stimulation ranged from 0 to +1 mV for the IHC's in this study. In other experiments, the possibility of a COCB synapse directly onto IHC's was ruled out by demonstrating that the hair cell membrane resistance did not change during COCB stimulation (*12*). Nonsensory cells often showed small depolarizations with COCB stimulation, and the extracellular potential within the organ of Corti in the inner hair cell region was always increased (up to 2 mV) during COCB stimulation. This may be a field potential resulting from the COCB action on OHC's [J. Fex, *J. Acoust. Soc. Am.* **41**, 666 (1967)] and it occurred for electrical pulse trains of either polarity, demonstrating that it was not a stimulus artifact. In addition, the gain of the recording system was unaffected by COCB stimulation.
12. M. C. Brown, thesis, University of Michigan (1983); _____ and A. L. Nuttall, in preparation.
13. M. L. Gifford, thesis, Massachusetts Institute of Technology (1979).
14. D. Ishii and K. Balogh, Jr., *Acta Oto-Laryngol.* **66**, 282 (1968); J. J. Guinan, W. B. Warr, B. E. Norris, in preparation.

15. K. Hama, *J. Cell Biol.* **24**, 193 (1965).
16. A. Flock and I. Russell, *J. Physiol. (London)* **257**, 45 (1976).
17. J. F. Ashmore and I. J. Russell, *ibid.* 329, 25P (1982); J. J. Art, A. C. Crawford, R. Fettiplace, P. A. Fuchs, *Proc. R. Soc. London Ser. B* **216**, 377 (1982).
18. R. V. Harrison and E. F. Evans, *INSERM* **68**, 105 (1977).
19. P. Dallos and D. Harris, *J. Neurophysiol.* **41**, 365 (1978).
20. J. Wersall, in *Basic Mechanisms in Hearing*, A. Møller, Ed. (Academic Press, New York, 1973), p. 235; J. E. Hawkins, Jr., *INSERM* **68**, 327 (1977).
21. H. Spoendlin, in *Facts and Models in Hearing*, E. Zwicker and E. Terhardt, Eds. (Springer-Verlag, New York, 1974), p. 18.
22. D. C. Mountain, *Science* **210**, 71 (1980); J. H. Siegel and D. O. Kim, *Hearing Res.* **6**, 171 (1982).
23. We thank D. Goodman for providing the technical details for staining IHC's and K. Anderson for providing literature concerning brain histology. Supported by NIH grants NS-05785, NS-15107, NS-07106, and NS-11731. Submitted by M.C.B. in partial fulfillment of the requirements for the Ph.D. thesis, University of Michigan.
* Present address: Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston 02114.

22 March 1983; revised 10 June 1983

Segregation and Mapping Analysis of Polymorphic HLA Class I Restriction Fragments: Detection of a Novel Fragment

Abstract. *An HLA-B7 complementary DNA clone was used as a hybridization probe to analyze the segregation pattern of polymorphic class I restriction fragments in several families whose HLA types had been determined by serological techniques. In one family in which a crossover in the HLA region had occurred, a specific genomic fragment was mapped with respect to the crossover. In another family, a novel genomic fragment present in one child and absent in all other family members was observed. With the exception of this novel fragment, all polymorphic class I fragments observed in this study segregated with a serologically defined parental haplotype, a result consistent with HLA linkage.*

The human major histocompatibility complex (MHC) is a highly polymorphic genetic region (HLA) on the short arm of chromosome 6; this region encodes products involved in various immunological functions [reviewed in (*1*)]. The class I genes encode the serologically defined transplantation antigens, *HLA-A*, *B*, and *C* as well as the human analogs to the murine Qa and T1 antigens (*2*, *3*). The transplantation antigens—composed of a polymorphic glycoprotein of 45,000 daltons associated with an invariant polypeptide of 12,000 daltons, β-2 microglobulin, which is encoded on chromosome 15—are present on the surface of all nucleated cells. Population studies have shown significant associations—and family analyses have demonstrated linkage—between specific serologically defined HLA antigens and a large number of human diseases [reviewed in (*4*)]. The genetic complexity of the class I genes revealed by recombinant DNA analysis is significantly greater than that predicted by serological analysis (*5*, *6*), with estimates of more than 30 class I genes in the mammalian MHC. Consequently, the identification and characterization of DNA sequence polymorphisms in the HLA region within normal (*7*, *8*) and diseased populations and their relation to the HLA alloantigens defined by classical serological and immunochemical techniques is of interest. DNA sequence polymorphisms can be detected as polymorphisms in the length of restriction fragments. We report the segregation analysis of polymorphic class I restriction fragments in two families. The HLA-B7 complementary DNA (cDNA) clone (*9*) was used as a hybridization probe. In one family, in which a crossover in the HLA region had occurred, a specific genomic fragment was mapped with respect to the crossover; in another family, a new fragment present in one child and absent in all other family members was observed.

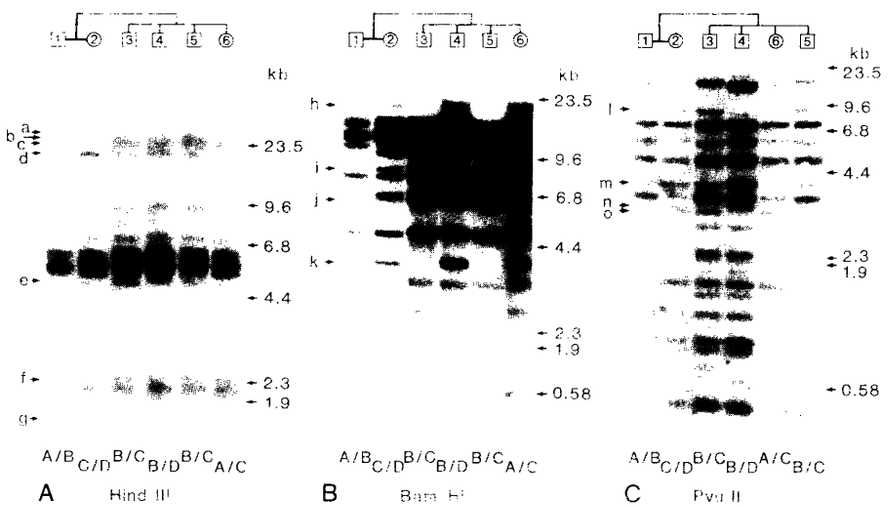

Fig. 1. Genomic blots of restriction endonuclease–digested DNA from an HLA-typed family hybridized with the HLA-B7 cDNA probe (*9*). High molecular weight DNA was prepared from approximately 2×10^7 frozen lymphocytes derived from blood samples of the individual family members as described (*12*). Each genomic DNA was digested with the restriction endonucleases Hind III (A), Bam HI (B), and Pvu II (C); subjected to electrophoresis; transferred to nitrocellulose (*14*, *15*); and hybridized with the nick-translated probe (*16*) as described (*17*). The filters were washed at 20°C in double-strength standard saline citrate and 0.1 percent sodium dodecyl sulfate, and subsequently at 50°C in 0.1-strength standard saline citrate and 0.1 percent sodium dodecyl sulfate, and analyzed by autoradiography. Polymorphic restriction fragments are denoted *a* to *o*. The large arrows represent specific size markers (Hind III digest of λ DNA). The HLA haplotype of the father [A2, C(−), Bw50 (Bw6), DR3, BfS1, Glo1/A11, Cw6, B37 (Bw4), DR5, BfS, Glo1) is designated *A/B* and of that of the mother [A3, C(−), B18 (Bw6), DR3, BfF1, Glo2/A25, C(−), B7 (Bw6), DR2, BfS, Glo1], *C/D*. The fragment n appears in the DNA of only one child and is absent from both parental DNA's. The print displayed in (A) is a composite of two different autoradiograms of the same blot; longer exposure time (bottom) was necessary to reveal the smaller fragments.

Genomic DNA from members of family Stk was digested with Hind III, Bam HI, and Pvu II; transferred to nitrocellulose; and hybridized with the ^{32}P-labeled HLA-B7 probe (Fig. 1). All autoradiographic bands clearly showing polymorphism were denoted by a lowercase letter and are listed in Table 1 along with the segregation pattern and inferred HLA haplotype assignments. Three different segregation patterns could be distinguished. In pattern 1 (exemplified by fragment a), a fragment present in DNA from only one parent appears in the DNA of some but not all of the children, allowing assignment to a unique parental haplotype. In pattern 2 (exemplified by fragment b), a fragment present in DNA from only one parent is present in the DNA of each child; this pattern is consistent with derivation of the fragment from both chromosome 6 homologs of the parent (that is, the mother is homozygous for the fragment, which segregates with both maternal haplotypes). In pattern 3 (exemplified by fragment h), a fragment is present in the DNA of both