that this "new ground" came into existence as a Mesozoic accretionary foldbelt but did not exist when Antarctica was attached to Africa (*8*).

Largely because of the two broad indentations in the eastern coast of Africa, drift proponents have suggested that the subcontinent of Madagascar, under drift reconstruction, must belong either against Mozambique (the southern position) or against Tanzania (the northern position). If our fit is valid, the Mozambique position for Madagascar is eliminated because in this position Madagascar would largely overlap Antarctica.

We suggest the following geologic history in terms of sea floor spreading and plate tectonics: Antarctica was initially split away from Africa in the late Triassic with the formation of the Indian-Antarctica rift. The initial rifting was probably synchronous with the emplacement of the Stormberg lavas and associated intrusives of Rhodesia, South Africa, and Swaziland, which yield mid-Triassic (−200 million years) to early Jurassic ages (*9*). Subsequently, Antarctica has remained quite fixed in latitude but has undergone westward rotation; Africa moved northward and rotated sinistrally. At the end of the Jurassic, the South Atlantic rift split South America away from Africa. Subsequently, both South America and Antarctica have moved westward and the accretion of the Mesozoic foldbelts, including the development of the horn of Antarctica, was associated with this westward drift (*10*).

Antarctica is entirely surrounded by the Pan Antarctica rift system ("mid-ocean ridge"), which probably also accommodates some shearing. There is no trench, or zone of crustal resorption, associated with the Antarctica plate toward which this continent might be expected to drift. Presumably, after initial blocking out of the margins of Antarctica, the Pan Antarctica rift system moved outward, causing the plate to grow larger. The rude circularity of this rift system is consistent with a tight rotation of the plate about a pole of rotation within Antarctica; this direction must have been westward, following the motion of South America. In terms of lining up the foldbelts of South America and Antarctica, the horn of Antarctica now lags about 15° in longitude behind South America. On the other hand, this horn has rotated 75° in longitude with respect to Africa.

We conclude that the best fit position shown in Fig. 1, which places western East Antarctica against southeastern Africa, is a valid continental drift reconstruction prior to mid-Triassic within the overall framework of the supercontinent of Gondwana.

*Note added in proof*: Since submission of this report, Smith and Hallam (*11*) have presented an Africa-to-Antarctica fit quite similar to ours. However, by using the 500-fathom isobath to delimit the continent edge, a somewhat different solution results. Notably, much of the Weddell Sea becomes a "mediterranean sea," whereas this area is land in our reconstruction, in which the 1000-fathom isobath was used.

ROBERT S. DIETZ
WALTER P. SPROLL
*Atlantic Oceanographic and Meteorological Laboratories, Environmental Science Services Administration, Miami, Florida 33130*

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7. A. L. Wegener placed Antarctica against southern Africa in more or less our position [*The Origin of Continents and Oceans* (Dover, New York, 1929; ed. 4, 1966)], but his sketch map is too imprecise and generalized to permit recognition of any antarctic landmarks. S. W. Carey juxtaposed Antarctica against eastern Africa but at a position considerably farther north than ours [in *Continental Drift, a Symposium*, S. W. Carey, Ed. (Geology Dept., Univ. of Tasmania, Hobart, Australia, 1958), pp. 177–355]. King rotated Antarctica relatively westward to line up the antarctic peninsula with the Andean foldbelt, as did Van Hilten 6 years later [L. C. King, *ibid.*, p. 65; D. Van Hilten, *Tectonophysics* **1**, 3 (1964)]. A. Holmes placed central East Antarctica against southeastern Africa [in *Principles of Physical Geology* (Ronald, New York, ed. 2, 1965), pp. 1193–1250]. F. Ahmad ["Paleogeography of the Gondwana period in Gondwanaland," *Mem. Geol. Surv. India 90* (1961)] and E. Irving [*Paleomagnetism and Its Application to Geological and Geophysical Problems* (Wiley, New York, 1964), pp. 256–273] juxtaposed East Antarctica (Queen Maud Land) against southeastern Africa. J. T. Wilson also placed central East Antarctica and Madagascar against southeastern Africa [*Nature* **198**, 925 (1963)]; this reconstruction results from directly closing the two cratons without any differential rotation and thus involves "least work." A. L. Du Toit lined up the Scotia Arc with the Cape ranges, lined up both with his Samfrau geosyncline, and left a small ocean basin off southeastern Africa [*Our Wandering Continents* (Oliver & Boyd, Edinburgh, 1937)].
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15 January 1970

## Cochlear Nerve Fiber Discharge Patterns: Relationship to the Cochlear Microphonic

Abstract. *Fourier analysis of discharge patterns in response to sinusoidal acoustic stimulation provides a consistent and repeatable measure of response phase and amplitude. The variation of the fundamental and harmonic components of the patterns as stimulus parameters are changed is strikingly similar to that of cochlear microphonics. The results are significantly different for single fibers with different characteristic frequencies; the variations parallel those of microphonics recorded from different cochlear turns.*

Frequency components of cochlear nerve fiber discharge patterns for sinusoidal stimulation show strong similarities in both phase and magnitude to results reported for the cochlear microphonic (CM) (*1*, *2*). As a result, the possibility that the CM is merely an epiphenomenon now seems unlikely. Furthermore, our findings support the interpretation (*2*) that harmonics of the stimulating frequency are generated locally along the basilar membrane and are not propagated throughout the cochlea.

Adult cats were anesthetized (Dial in urethane, 0.75 mg/kg), and the cochlear nerve was exposed. In a sound-quieted room the animals were stimulated with continuous sine waves from a low-distortion acoustical system (*3*). The bony septum between the bulla and the middle ear cavities was removed to eliminate acoustical resonant effects (*4*). Spike discharges were monitored with KCl-filled micropipettes. Period histograms (*5*) were computed on a Laboratory Instrument Computer (LINC), each for a fixed duration of stimulation. A fast Fourier transform technique (*6*) was applied to the period histograms to obtain the amplitudes and phases of the fundamental

component ($f_1$) and the first two harmonic components ($f_2$ and $f_3$) of the discharge patterns.

The phase of the fundamental component of the discharge pattern as a function of stimulus frequency is plotted for 11 fibers in Fig. 1. These data are typical of our results on about 45 nerve fibers from nine different animals in that two straight lines closely approximate the data points. The fibers represented in Fig. 1 vary in their characteristic frequency (CF) (*7*) over a range of 5000 hz. The slopes of the curves monotonically decrease with increasing CF. For fibers with low CF (less than about 1000 hz) the rate of change of phase is greater at low frequencies than at high, and the opposite is true for fibers with high CF (above about 1200 hz). For a given fiber the frequency at which the slope changes is usually close to the CF, especially when the CF is below about 4000 hz. The degree of change of the slope tends to increase as the CF departs in either direction from the 1000- to 1200-hz range. Fibers with CF's of about 1100 hz show little or no deflection in their phase characteristics.

The heavy dashed lines (Fig. 1) are derived from measurements of CM in the guinea pig (*1*). The phase characteristics of the CM measured at different turns in the cochlea show that they can also be approximated by two straight lines; their break points are also near the frequency of maximum sensitivity; and the directions of the deflections are also in one direction for low-frequency regions (turns III and IV), and in the opposite direction for the higher frequency region (turn II). For *both sets of data* extrapolations of the straight-line approximations do not necessarily intercept the origin, and the intercepts of the extrapolations generally move to the right of the origin as CF increases or turn number decreases.

Relative amplitudes of $f_1$, $f_2$, and $f_3$ of the discharge patterns plotted as a function of stimulus frequency for one fiber are shown in Fig. 2. All the functions show maximum amplitude at approximately the same stimulus frequency. We have seen no exception to this result, and it is similar to data from the CM (*2*). These data furnish specific support for arguments (*2, 8*), based on CM data, that harmonic components of distortion are generated in the region of the basilar membrane most sensitive to its eliciting fundamental component,

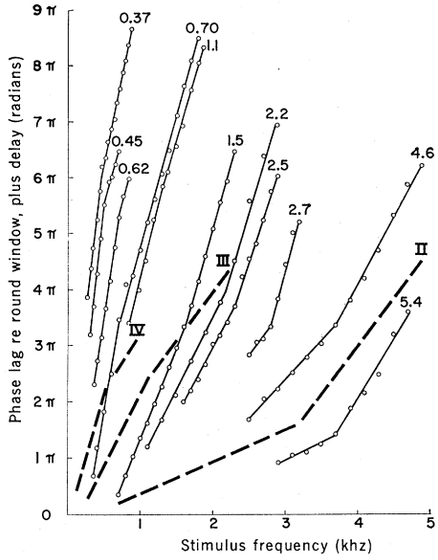
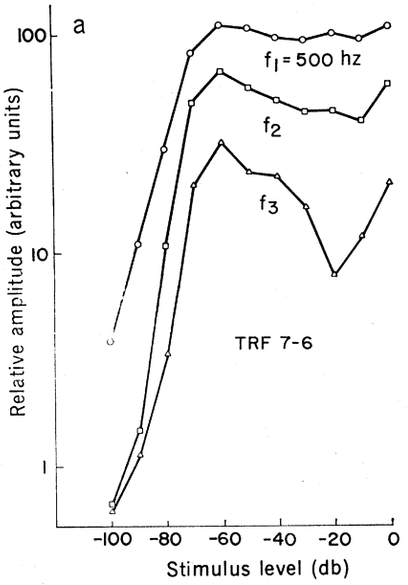
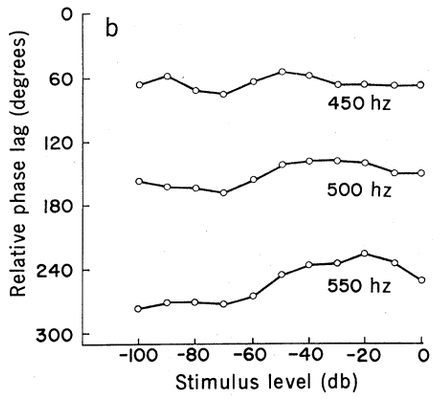



Fig. 1 (left). Plots of phase shift of the fundamental of the discharge patterns (corrected to the round window potential) as a function of stimulus frequency (*9*). Each solid curve is for a single cochlear nerve fiber; the CF (*7*) of the fiber in kilohertz is specified above each curve. The dashed curves are for CM measured from cochlear turns II, III, and IV (*1*) of the guinea pig, also relative to the round window potential. The signal level for any one curve was held constant. The signal level has little effect on phase (see Fig. 3b), except occasionally at very high stimulus levels (about 90 to 100 db relative to 0.0002 dyne per square centimeter; these results are not reported here).

Fig. 2 (right). Relative amplitudes of $f_1$, $f_2$, and $f_3$ as a function of stimulus frequency for one single fiber. The signal level was approximately + 40 db relative to 0.0002 dyne per square centimeter.



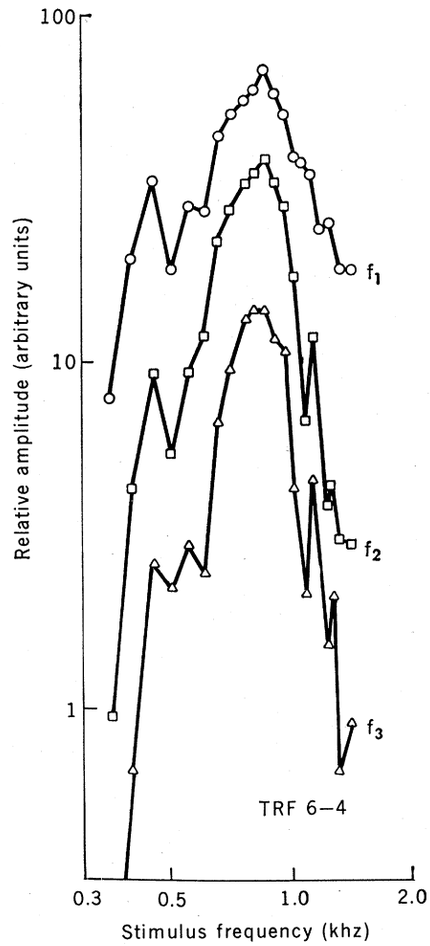




Fig. 3. (a) Relative amplitudes of $f_1$, $f_2$, and $f_3$ as a function of signal level in decibels; 0 db is approximately 100 db relative to 0.0002 dyne per square centimeter. The CF of this fiber was about 515 hz. (b) Relative phase of $f_1$ as a function of signal level for three different frequencies. Typically, phase is affected little over the range from −100 to −50 db. Often, although it is not seen here, there is a drastic shift at approximately −10 db.

in contrast to the possibility that the harmonics are present entirely as a traveling wave within the cochlea.

Relative amplitudes of $f_1$, $f_2$, and $f_3$ plotted as a function of signal level for one unit are shown in Fig. 3a. For this unit, and generally, (i) the amplitude of $f_1$ is proportional to stimulus amplitude at low stimulus levels; (ii) the amplitude of $f_2$ is greater than that of $f_3$; and (iii) the amplitudes of $f_2$ and $f_3$ rise more rapidly than that of $f_1$ at low signal levels. Each of these properties corresponds to observations of amplitude characteristics of harmonics of CM (*2*). Figure 3b shows the relative phase plotted as a function of signal level of the fundamental component for three different frequencies for the same unit as that shown in Fig. 3a. Changes of phase of less than 20 degrees over dynamic ranges of 50 to 60 db are not uncommon. The amplitude and phase correspondences reported above suggest that there is a very close relationship between the CM and the discharge activity of cochlear nerve fibers.

RUSSELL R. PFEIFFER
CHARLES E. MOLNAR
*Washington University, Box 1127, St. Louis, Missouri 63130*

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9. It is well known that for click stimulation the microphonic of the round window potential occurs ahead of the $N_1N_2$ component. Therefore, in an attempt to obtain quantitative agreement between CM and single fiber results, we have incorporated in the plots of Fig. 1 an estimated 0.5 msec delay between initiation of the CM and the initiation of the spike discharges. (To change back to the original single fiber results, one merely adds $1.0\pi$ of the lag per kilohertz). The differences remaining are perhaps due to the difference in species.
10. Supported by PHS research grants NS 07498, FR-396, FR-504, and FR-6115 from the National Institutes of Health. We thank K. Williams, M. A. Kelly, R. Cox, and T. DeWoskin for their assistance and support.

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## Preparation of Beating Heart Cells from Adult Rats

Abstract. *Suspensions of cardiac cells have been prepared from fragments of hearts from adult rats by prolonged incubation with trypsin and collagenase. In these preparations, about 50 percent of the viable cells routinely exhibit spontaneous contractions ranging from 20 to 120 beats per minute at room temperature. The contractile activity is essentially lost at 2°C but returns to normal as the temperature is increased. The rate of cellular contractions and the percentage of total cells exhibiting contractility are influenced by experimental conditions of cell preparation and the composition of the suspending medium.*

The routine preparation of component cells from various tissues can be accomplished by tissue perfusion or incubation with a variety of proteolytic enzymes. In 1955, Cavanaugh (*1*) reported on the preparation of suspensions of heart cells obtained by incubation of neonatal chick heart (5 days old) with trypsin. However, spontaneous contractions were observed in only a portion of the cells and only after several hours, when the cells attached to the glass culture tube. Similarly, individual cells isolated from heart tissue of neonatal rats exhibit spontaneous beating after attachment of the cells to the glass surface of the culture vessel (*2*). These cells continue to beat in culture as they increase in size, and, as the cells come into contact with each other, they begin to beat in synchrony (*1*, *3*). Harary (*4*) recently reviewed the various factors influencing contractility and certain metabolic activities of neonatal rat heart cells in culture.

Until now, there has not been a report of the preparation of spontaneously beating cells from adult mammalian tissue. Kono (*5*) recently reported on the preparation of a cell suspension from cardiac muscle tissue from adult rats but did not describe the viability of the cells. Harary and Farley (*3*) indicated that cells prepared from hearts of 6-week-old rats are relatively inactive, and those prepared from hearts of 16-week-old rats are essentially all spherical cells with no contractile properties.

The present report describes a procedure for preparation of cardiac cells from adult heart tissue and certain factors influencing contractility of these cells in dispersed suspensions.

Hearts from adult male rats (250 g) were dissected free of major blood vessels and atria and were minced into coarse fragments (3 mm) with a razor blade. These were incubated for 30 minutes at 34° to 36°C in saline A (*6*) containing 0.1 percent trypsin and 0.1 percent collagenase (2 to 3 ml per heart). The supernatant was discarded and the remaining tissue fragments were reincubated for 30 minutes in 2 to 3 ml of saline A solution containing 0.1 percent trypsin and 0.05 percent collagenase. At 30-minute intervals thereafter, the supernatants were collected and the residual tissue fragments were reincubated in fresh enzyme solution. Heart cells were obtained from the supernatants by gentle centrifugation, and these were resuspended in various media. Total number of cells and the percentage of total cells with contractile properties were determined by repeated counting of samples of the cell suspensions on hemocytometer slides. A qualitative measurement of viability of the nonbeating cells was obtained by vital staining with neutral phenol red.

The incubation of adult heart tissue fragments with either trypsin or collagenase alone was ineffective in yielding a large number of cardiac cells with spontaneous contractile behavior (Fig. 1). Cells obtained during the initial 30-minute incubation in the first enzyme solution described below consisted largely of blood cells and damaged cardiac cells. The total number of cells and the proportion of contracting cardiac cells progressively increased as the incubation was continued. Cell suspensions obtained from the 90- to 120-minute incubation contained primarily cardiac cells, of which approximately 80 percent were rod-shaped (Fig. 2) and of these 40 to 60 percent exhibited spontaneous contractions ranging from 20 to 120 beats per minute. This same incubation procedure has been applied successfully to the preparation of contracting cardiac cells from adult human heart (*7*).

The cells generally range in size from 10 to 50 $\mu$m in diameter and from 50