of the diverse effects of interferon and together they suggest some new, testable hypotheses for the ability of interferon to inhibit tumor spread (*14*). For example, if interferon were able to prevent neovascularization of growing tumors in vivo by preventing the requisite migration of capillary endothelial cells (*15*), the relative lack of vessels could not only slow tumor growth by limiting nutrient supply but might also inhibit tumor spread by limiting contact with the vascular tree. Second, if the motility-inhibitory activity of interferon should prove applicable to the migration of tumor cells in vivo, interferon might serve to immobilize the tumor cells themselves and directly prevent their spread. Thus the efficacy of interferon as an antitumor agent may depend upon its ability to inhibit cell migration in the host.

DANIÈLE BROUTY-BOYÉ*
BRUCE R. ZETTER†
Departments of Surgery and *Medical Microbiology and Molecular Genetics, Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts 02115*

References and Notes

1. I. Gresser, *Cell. Immunol.* **34**, 406 (1970); ——— and M. G. Tovey, *Biochim. Biophys. Acta* **516**, 231 (1978); J. L. Marx, *Science* **204**, 1183 (1979); *ibid.*, p. 1293.
2. C. Baglioni, *Cell* **17**, 255 (1979).
3. G. Albrecht-Buehler, *ibid.* **11**, 395 (1977); *J. Cell Biol.* **72**, 595 (1977).
4. B. R. Zetter, *Nature (London)*, in press.
5. I. Gresser, M.-T. Bandu, D. Brouty-Boyé, M. G. Tovey, *Nature (London)* **251**, 543 (1974).
6. K. E. Mogensen and K. Cantell, *Pharmacol. Ther. A* **1**, 369 (1977).
7. J. Folkman, C. Haudenschild, B. R. Zetter, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5217 (1979).
8. The method for determination of track size is described in the legend to Fig. 2. In this experiment, the cultures were fixed with Formalin to terminate the experiment 18 hours after the addition of tumor-conditioned medium, and only those tracks made by single cells were analyzed. This was done to eliminate consideration of the effect of cell-cell collision and cell division on cell motility.
9. Rabbit antiserum against human leukocyte interferon was obtained from the NIAID Antiviral Substances Program, National Institutes of Health. A 1:640 dilution of the serum neutralized 32 units of human leukocyte interferon, as assayed on both human skin fibroblasts and BCE cells inoculated with vesicular stomatitis virus.
10. In the absence of interferon, BCE cells grew from 0.25×10^5 to 5.0×10^5 cells per square centimeter in 6 days in tumor-conditioned medium. In the presence of 64 antiviral units of interferon, cell density reached only 1.7×10^5 cells per square centimeter.
11. A. Billiau, J. Van Damme, F. Van Leuven, V. G. Edy, M. De Ley, J. J. Cassiman, H. Van Den Berghe, P. De Somer, *Antimicrob. Agents Chemother.* **16**, 49 (1979).
12. Antiviral titers for interferon are expressed as the reciprocal of the dilution that protects 50 percent of the cell sheet against a challenge of 100 median infective doses of vesicular stomatitis virus.
13. M. G. Tovey, J. Begon-Lours, I. Gresser, *Proc. Soc. Exp. Biol. Med.* **146**, 809 (1976).
14. I. Gresser and C. Bourali, *Nature (London) New Biol.* **236**, 78 (1972); H. Strander, *Blut* **35**, 277 (1977); T. C. Merigan *et al.*, *N. Engl. J. Med.* **299**, 1449 (1979).
15. D. H. Ausprunk and J. Folkman, *Microvasc. Res.* **14**, 53 (1977).
16. We thank Drs. K. Cantell, J. Vilček, and I. Gresser for their gifts of interferon and Dr. M. Levin for the rabbit antiserum against human leukocyte interferon. This work was supported by grant 1496-C1 from the American Cancer Society, Massachusetts Division, and by grant R01-Cal4019 from the National Institutes of Health to Dr. J. Folkman, whom we thank for his generous support. The Zeiss MOP-3 digital image analyzer was provided by a grant to Harvard University from the Monsanto Company.

B.R.Z. is a Glick-Franzheim cancer research fellow at Children's Hospital Medical Center.
* Visiting scientist on leave from the Laboratoire d'Oncologie Virale, Institut de Recherches Scientifique sur le Cancer, BP No. 8, 94800, Villejuif, France.
† Address requests for reprints to B.R.Z.

17 October 1979, revised 5 December 1979

Hearing in the Elephant (*Elephas maximus*)

Abstract. *Auditory thresholds were determined for a 7-year-old Indian elephant. The hearing range extended from 17 hertz to 10.5 kilohertz. The results indicate that the inverse relationship between functional interaural distance (that is, the distance between the two ears divided by the speed of sound) and high-frequency hearing limit is valid even for very large mammals.*

Among vertebrates, only mammals can hear high-frequency sounds (*1*). Whereas other classes of vertebrates are unable to hear much above 10 kHz, the average mammalian high-frequency hearing limit is 55 kHz, and high-frequency limits near 100 kHz are not uncommon. Yet the ability of mammals to hear high frequencies is not uniform, but varies from one species to the next. For example, humans are generally capable of hearing 19 kHz, dogs 44 kHz, rats 72 kHz, and bats 115 kHz (*2*). Thus, high-frequency hearing among mammals varies over a range of nearly three octaves.

At first the variation in mammalian high-frequency hearing was thought to be related to the size of the animal, as small mammals seem better able to hear high-frequency sounds than larger ones (*3*). More recently, however, it has been shown that high-frequency hearing is directly correlated not with body weight, but with the functional distance between the two ears, where functional distance (Δt) is defined as the distance between the ears (interaural distance) divided by the speed of sound (*1*). Mammals with small heads, and therefore close-set ears, are better able to hear high-frequency sounds than species with large heads and wide-set ears. More precisely, high-frequency hearing varies inversely with the functional distance between the ears and ultimately with the interaural time and intensity difference cues used for sound localization. Thus, the variation in mammalian high-frequency hearing is neither random nor, on the whole, the result of adaptations to specialized habitats—even those such as of bats or dolphins. Instead, high-frequency hearing seems to vary predictably with interaural distance.

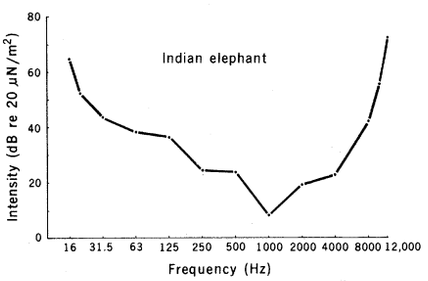

Fig. 1. Audiogram of the elephant. The thresholds represent the average of two audiograms, one obtained with the loudspeaker on the animal's left and the other with the loudspeaker on the right. Frequencies were tested in octave steps from 16 Hz to 8 kHz. Additional frequencies were 20 Hz, 10 kHz and 12 kHz. Sound pressure levels were recorded at the position of the opening of the elephant's auditory canal with and without the elephant in the sound field. To make the results comparable with previous audiograms, the thresholds reported here are based on measurements without the elephant in the sound field. (Sound pressure levels with the animal in the sound field were on average 2 dB higher, with the largest increase being 5 dB.)

The relationship between functional interaural distance and high-frequency hearing, however, has been established primarily for animals with small interaural distances. While there is no reason not to believe that this relationship is equally valid for all mammals, extrapolation to very large mammals leads to two somewhat unexpected conclusions. First, it predicts that a mammal as large as an elephant would hear sound only to about 10 kHz. Since this limit is no higher than that found in birds, this prediction suggests that mammalian high-frequency hearing may not always be superior to nonmammalian vertebrates. Second, humans have often been considered as aberrant because of their inability to hear above 20 kHz (*1*, *4*). However, it would now appear that humans may not be unusual in this respect, but may even have better high-frequency hearing than certain other (larger) mammals. Thus, to determine if the relationship applied to very large mammals we decided to test the hearing of an elephant.

The elephant chosen for this study was a 7-year-old (adolescent) female Indian

0036-8075/80/0502-0518$00.50/0

elephant (*Elephas maximus*) with no history of serious illness. Her ears were carefully inspected and found to be free of any signs of obstruction or disease.

The audiogram was determined according to a two-choice or yes-no procedure, in which the elephant indicated the presence or absence of a tone by making one response when a tone was perceived and a different response when it was not perceived (*5*). Specifically, a panel (70 by 50 cm) had three Plexiglas response buttons (13 cm in diameter) mounted at the top in a horizontal row and a small drinking trough (13 by 15 by 7 cm) located at the bottom of the panel directly below the center button. The response panel was mounted on a cement-block wall 1.6 m high and topped by a chain fence. This wall and fence separated the elephant's room (6.7 by 5.6 by 4.6 m) from an adjacent room housing the test equipment. A loudspeaker was located 1 m to either the left or right side of the response panel and was pointed toward the elephant's ears (*6*).

The elephant was tethered 1.2 m in front of the response panel and trained to press the center button with its trunk. This response constituted an "observing response" which initiated a trial and also positioned the animal's head directly in front of the panel. Once a trial had begun, the elephant was required to wait at least 2 seconds and then press the left button if a tone had been presented or the right button if no tone had been presented. A correct response was rewarded with 30 ml of fruit-flavored drink dispensed into the trough. An incorrect response was not rewarded and was followed by a 5-second wait before a new trial could be begun. Tone pulses were presented randomly on half of the trials, and thresholds were obtained by presenting tones of various intensities (according to the method of constant stimuli). Threshold was defined as the intensity at which the animal could just distinguish tone trials from no-tone trials at the .01 level of statistical reliability (binomial distribution) for 50 trials (*7*). Testing was judged complete for a particular frequency when thresholds from two different sessions were within 3 dB of each other.

The elephant's audiogram exhibits the characteristic shape of mammalian audiograms (Fig. 1) (*1*). Beginning at the low frequencies, threshold decreased gradually to a relatively well defined point of best hearing in the midfrequency range followed by a steep increase in threshold at the high frequencies. The elephant's sensitivity was about average; the lowest threshold, 8-dB sound pressure level at 1 kHz, was well within the range of "best sensitivities" for mammals.

In spite of these similarities, the elephant's audiogram differs from those of other mammals in two major ways. (i) The elephant was unable to hear above 10.5 kHz at an intensity level of 60 dB. Though slightly higher frequencies could be heard at very high intensities, the animal was completely unable to respond to frequencies above 12 kHz at an intensity of 90 dB. (ii) The elephant was able to hear low-frequency sounds better than any mammal previously tested. At an intensity of 60 dB, the elephant could hear 17 Hz, nearly one octave below the comparable human threshold of 29 Hz. In sum, the elephant's audiogram was typically mammalian in form, but shifted toward low frequencies.

The audiogram of the elephant demonstrates the validity of the relationship between interaural distance and high-frequency hearing for all mammals large or small, land or water, echolocators or not (Fig. 2) (*1*). Where interaural distance is represented by maximum Δt, that is, the interaural distance divided by the speed of sound (*8*), the correlation between maximum Δt and the high-frequency hearing limit is $-.89$ ($P < .001$). This correlation is now based on audiograms for 32 genera ranging from mouse and bat to elephant and killer whale. This high correlation implies that about 80 percent of the variance in the upper limit of hearing is accounted for by the variance in functional interaural distance alone.

The existence of a strong inverse relationship between these variables has been ascribed to selective pressure for accurate sound localization (*1*). Briefly, the two binaural cues for sound localization, the difference in time of arrival of a sound at the two ears (Δt) and the difference in frequency-intensity spectra of a sound reaching the two ears (Δfi), depend on the functional distance between the two ears and the sound shadow of the head and pinnae. That is, the farther apart the ears, the larger will be the Δt cue for any given direction of a sound source. Similarly, the Δfi cue is greater for animals with wide-set ears both because the sound attenuation is slightly greater over the longer distance between the ears and because animals with wide-set ears usually have large heads or large pinnae which effectively shadow the high-frequency content of sound. While the two binaural sound-lo-

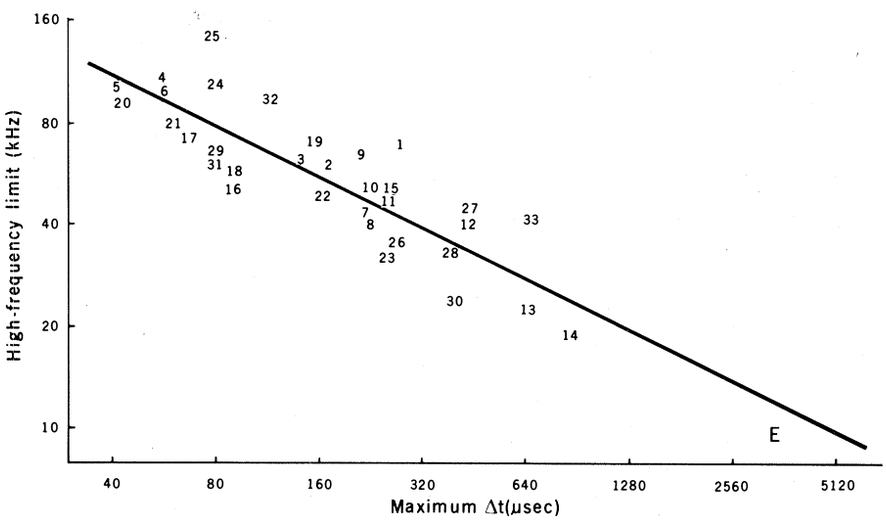

Fig. 2. Relationship between maximum Δt (maximum interaural distance divided by the speed of sound) and high-frequency hearing limit (highest frequency audible at 60-dB sound pressure level). Numbers and letter represent points for individual species (*9*). All high-frequency limits were determined in air except as noted. Key: *E*, elephant (*Elephas maximus*); 1, opossum (*Didelphis virginiana*); 2, hedgehog (*Hemiechinus auritus*); 3, tree shrew (*Tupaia glis*); 4, horseshoe bat (*Rhinolophus ferrumequinum*); 5, little brown bat (*Myotis lucifugus*); 6, big brown bat (*Eptesicus fuscus*); 7, slow loris (*Nycticebus coucang*); 8, potto (*Perodicticus potto*); 9, bush baby (*Galago senegalensis*); 10, owl monkey (*Aotus trivirgatus*); 11, squirrel monkey (*Saimiri sciureus*); 12, macaque (*Macaca* sp.); 13, chimpanzee (*Pan troglodytes*); 14, human (*Homo sapiens*); 15, rabbit (*Oryctolagus cuniculus*); 16, kangaroo rat (*Dipodomys merriami*); 17, cotton rat (*Sigmodon hispidus*); 18, gerbil (*Meriones unguiculatis*); 19, laboratory rat (*Rattus rattus*); 20, feral house mouse (*Mus musculus*); 21, laboratory mouse (*Mus musculus*); 22, guinea pig (*Cavia porcellus*); 23, chinchilla (*Chinchilla* sp.); 24, dolphin (underwater) (*Inia geoffrensis*); 25, porpoise (underwater) (*Tursiops truncatus*); 26, killer whale (underwater) (*Orcinas orca*); 27, dog (*Canis familiaris*); 28, sea lion (in air) (*Zalophus californianus*); 29, harbor seal (underwater) (*Phoca vitulina*); 30, harbor seal (in air) (*Phoca vitulina*); 31, ringed seal (underwater) (*Pusa hispida*); 32, harp seal (underwater) (*Pagophilus groenlandicus*); and 33, domestic sheep (*Ovis aries*).

calization cues are readily available to animals with large heads, the effectiveness of either cue is diminished in animals with functionally close-set ears. In the case of Δt, the available time difference may be so small that the nervous system can detect only gross changes in sound direction. However, an animal with a small head always has a Δfi cue available, providing only that it is able to perceive frequencies which are high enough to be effectively shadowed by its head and pinnae. Therefore, given the ecological importance of an animal's localizing the sound of a stealthy intruder, animals with functionally close-set ears are subjected to more selective pressure to hear high frequencies than animals with more widely set ears.

Finally, the finding that the elephant is unable to hear significantly above 10 kHz has two immediate implications for ecological and evolutionary acoustics. (i) It suggests that when the selective pressure for high-frequency hearing is reduced as a consequence of evolving a large interaural distance, the upper limit of hearing is reduced to the point at which it does not greatly exceed that of nonmammalian vertebrates, such as birds, many of which hear up to 10 kHz. (ii) It appears that humans should no longer be considered aberrant among mammals for their lack of ability to hear above 20 kHz. Instead, restricted high-frequency hearing seems to be a consequence of a relatively large interaural distance and not the result of a special adaptation for the reception of speech sounds, as was once widely believed.

RICKYE HEFFNER
HENRY HEFFNER

Bureau of Child Research,
University of Kansas, Parsons 67357

References and Notes

1. B. Masterton, H. Heffner, R. Ravizza. *J. Acoust. Soc. Am.* **45**, 966 (1969); B. Masterton and I. T. Diamond, in *Handbook of Perception: Biology of Perceptual Systems*, E. C. Carterette and M. P. Friedman, Eds. (Academic Press, New York, 1973), vol. 3, pp. 407–482.
2. H. Davis, in *Hearing and Deafness*, H. Davis and S. R. Silverman, Eds. (Holt, Rinehart & Winston, New York, 1960), pp. 29–60; H. Heffner, *J. Acoust. Soc. Am.* **58**, 124 (1975); ——, R. VanOeveren, B. Masterton, *ibid.* **50**, 148 (1971); J. I. Dalland, *Science* **150**, 1185 (1965).
3. G. von Békésy. *Experiments in Hearing* (McGraw-Hill, New York, 1960), pp. 500–510; —— and W. A. Rosenblith, in *Handbook of Experimental Psychology*, S. S. Stevens, Ed. (Wiley, New York, 1951), pp. 1075–1115.
4. E. G. Wever, in *Handbook of Sensory Physiology*, W. D. Keidel and W. D. Neff, Eds. (Springer, Heidelberg, 1975), vol. 5, part 1, pp. 423–454.
5. B. Mohl, *J. Aud. Res.* **8**, 27 (1968).
6. Loudspeakers used were a 15-inch (38-cm) woofer in a 0.45-m^3 sealed enclosure and a piezoelectric tweeter. Sound production and measurement were by techniques described elsewhere [R. Heffner, H. Heffner, B. Masterton, *J. Acoust. Soc. Am.* **49**, 1888 (1971)].
7. Varying the definition of threshold over a wide range has no significant effect on the main conclusions.
8. Maximum Δt in a given species is the maximum possible difference in the time of arrival of a sound at the two ears. The value for maximum Δt depends on the path which the sound travels from ear to ear as well as the velocity of sound in the particular medium, that is, the distance from ear to ear divided by the speed of sound in air (340 m/sec) around the head in terrestial mammals; and the distance from ear to ear through the head divided by the speed of sound in water and tissue (1500 m/sec) in most marine mammals.
9. For a list of the audiograms, see R. L. Francis [in *Sound Reception in Mammals*, R. J. Bench, A. Pye, J. D. Pye, Eds. (Academic Press, London, 1975), pp. 237–289], H. Heffner and B. Masterson (*J. Accoust. Soc. Am.*, in press) and the sources in (*2*).
10. We thank the Ralph Mitchell Zoo of Independence, Kans., for allowing us to study their elephant, and N. Stichman, for his help in training her. Supported by NSF grant BNS 58-07391.

22 October 1979; revised 7 February 1980

Cadmium Concentrations in Blood

In their report on in vivo cadmium measurements, Ellis *et al.* state that they found no significant difference in mean blood plasma or urine concentrations of smokers as compared with nonsmokers (*1*). The plasma and urine cadmium concentrations for nonexposed individuals reported in (*1*) are at least three to five times higher than those reported in the current literature (*2*).

Studies of cadmium partitioning in whole blood from laboratory animals exposed to cadmium indicate that the major portion of the metal is contained in the erythrocytes (*3*). Thus, the whole blood cadmium concentration might be a more appropriate parameter to measure. For nonoccupationally exposed nonsmokers over 36 years ($N = 27$), Pleban and Pearson found the mean blood cadmium concentration to be 1.00 ± 0.48 μg/liter (arithmetric mean $\pm$ standard deviation), whereas smokers over 36 years ($N = 18$) had a mean blood cadmium concentration of 2.21 ± 0.92 μg/liter (*4*). These means are significantly different (t-test, $P < .001$) and are in agreement with similar findings of others (*5*).

The normally low body fluid concentrations of cadmium require that extreme care be taken in sample collection and analysis procedures. This is particularly true for analyses in which extraction and wet ashing procedures are used. Sporadic cadmium contamination of 0.5 to 1.0 μg/liter, which is not as critical when measuring higher cadmium concentrations in organs of the body, can mask significant differences between populations with body fluid concentrations at the microgram per liter level.

PATRICIA PLEBAN

Department of Chemical Sciences,
Old Dominion University,
Norfolk, Virginia 23508

References

1. K. J. Ellis, D. Vartsky, I. Zanzi, S. H. Cohn, S. Yasumura, *Science* **205**, 323 (1979).
2. C.-G. Elinder, T. Kjellstrom, L. Linnman, G. Pershagen, *Environ. Res.* **15**, 473 (1978); H. A. Roels, J.-P. Buchet, R. Lauwreys, P. Bruaux, F. Claeys-Thoreau, A. LaFontaine, J. van Overschelde, G. Verduyen, *ibid.*, p. 290; E. L. Arnold, S. B. Hawthorne, R. J. MacDonald, paper 246 presented at the 31st national meeting of the American Association for Clinical Chemistry, New Orleans, July 1979.
3. L. Friberg, M. Piscator, G. F. Nordberg, T. Kjellstrom, *Cadmium in the Environment* (CRC Press, Cleveland, Ohio, 1974).
4. P. Pleban and K. H. Pearson, *Clin. Chim. Acta*, in press.
5. R. L. Zielhuis, E. J. Stuik, R. F. M. Herber, H. J. A. Salle, M. M. Verbeck, F. D. Posma, H. H. Jager, *Int. Arch. Occup. Environ. Health* **39**, 53 (1977); A. Ulander and O. Axelsson, *Lancet* **1974-I**, 682 (1974).

6 September 1979

Pleban leaves the impression that our values are too high and due to contamination when in fact the blood cadmium concentrations for nonoccupationally exposed smokers and nonsmokers she cites are not statistically different from the plasma values we reported in (*1*). We agree that extreme care must be taken in the collection and analysis procedures, and the wide range of values reported in the literature renders interlaboratory comparisons difficult. Nevertheless, we concur with Pleban that cadmium concentrations in the blood of smokers are generally higher than those in the blood of nonsmokers. The P values (mean differences by the t-test) for our small sample population of smokers and nonsmokers were .07 and .06 for cadmium concentrations in the plasma and urine, respectively.

However, in our investigation we found no significant relationship between the plasma or urine data and the kidney or liver burden of cadmium. Recently, we have studied workers occupationally exposed to cadmium (*2*). Although blood and urine cadmium concentrations were marginally correlated on a group basis, they were not predictive of the cadmium burden in the kidney or liver of an individual.

KENNETH J. ELLIS

Medical Department,
Brookhaven National Laboratory,
Upton, New York 11973

References

1. K. J. Ellis, D. Vartsky, I. Zanzi, S. H. Cohn, S. Yasumura, *Science* **205**, 323 (1979).
2. K. J. Ellis, W. D. Morgan, I. Zanzi, S. Yasumura, D. Vartsky, S. H. Cohn, 2nd Annual Park City Environmental Health Conference, Park City, Utah, 27–29 March 1980.

10 March 1980

0036-8075/80/0502-0520$00.50/0