long periods against morphine lethality suggests that the high-affinity sites do not mediate the lethal effects, which presumably include respiratory depression and interference with cardiovascular function (*10*). The ability of naloxazone to differentiate between analgesia and opiate mortality suggests that different subpopulations of receptors mediate various pharmacological effects. The distinct high- and low-affinity receptors described here do not appear to correspond to mu, kappa, and sigma receptors identified on pharmacological grounds (*1*) or to enkephalin or morphine binding sites (*2*). Conceivably, opiates with affinities for certain subpopulations of receptors might be selected to elicit analgesia without respiratory depression.

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5 December 1979

Inhibition of Cell Motility by Interferon

Abstract. *Interferon derived from human leukocytes, human fibroblasts, and mouse fibroblasts was found to inhibit the motility of cultured cells. It inhibits the tumor-induced motility of capillary endothelial cells as well as the spontaneous migration of other cell types. The ability of a given preparation of interferon to inhibit the motility of a given cell type is proportional to its antiviral activity in that particular cell type. Antiserum to human leukocyte interferon neutralizes both the motility-inhibitory activity and the antiviral activity of this preparation.*

Although interferon originally captured interest as an antiviral agent, it has become increasingly clear that this molecule can regulate other biological activities. Interferon inhibits cell multiplication in vivo and in vitro, modifies reactions of the immune system in vivo, and is currently being tested as an antitumor agent in animals and humans (*1*). Whereas much is known about the mechanism of interferon's antiviral activity (*2*), the mechanism by which it exerts antitumor activity is unclear.

Recently, in an effort to study the way in which new capillaries are induced to migrate toward a growing tumor, we developed a quantitative in vitro assay to study the motility of cultured cells. Analysis of cell motility is performed by measuring the dimensions of the phagokinetic tracks (*3*) left by cells as they move across gold-coated cover slips. It has been found that tumor cell extracts dramatically stimulate the phagokinetic movement of capillary endothelial cells (*4*). We report here that interferon

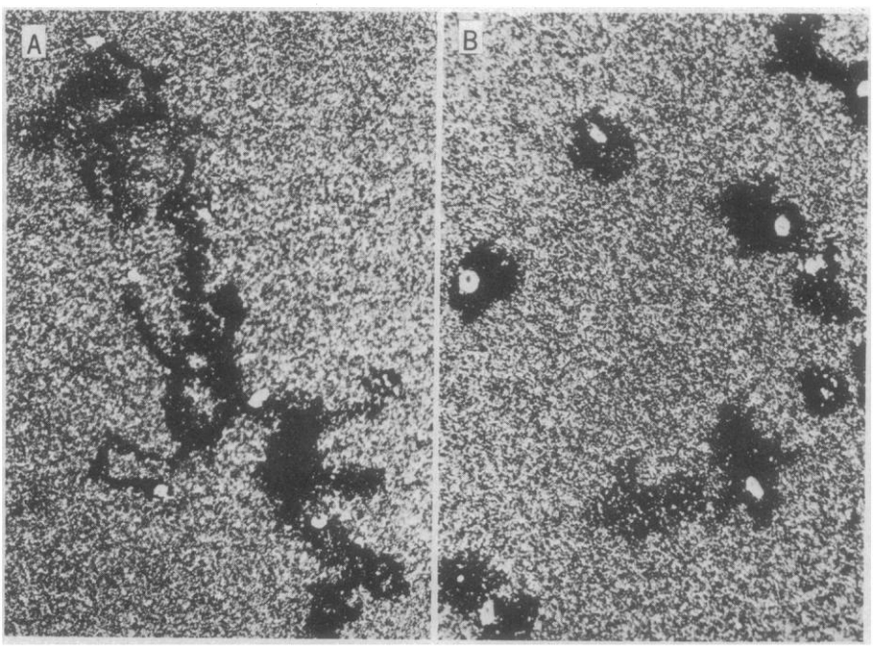

Fig. 1. Inhibition of tumor-stimulated bovine capillary endothelial cell migration by human leukocyte interferon. Bovine capillary endothelial cells (3000) were seeded onto gold-coated glass cover slips (22 mm square) prepared according to the method of Albrecht-Buehler (*3*). The cultures were incubated at 37°C in sarcoma-conditioned Dulbecco's modified Eagle medium containing 10 percent calf serum (A) or in the same medium plus 64 antiviral units of human leukocyte interferon (1.5×10^6 units per milligram of protein) (B) (*6*). After an additional 36 hours, the medium was removed and replaced with 2.5 ml of 10 percent phosphate-buffered Formalin (Fisher Scientific) to terminate the experiment. The phagokinetic tracks were observed under incident light with a Nikon MS inverted microscope and photographed with a Wild Heerbrugg MKA4 photoautomat camera (magnification, × 150).

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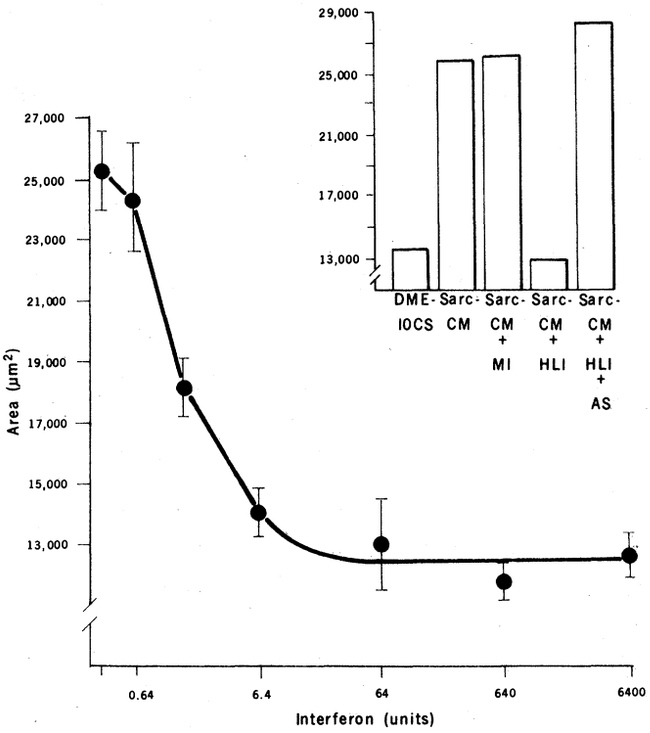

Fig. 2. Dose response of the effect of human leukocyte interferon on BCE cell migration. Bovine capillary endothelial cells (3000) were seeded on gold-coated cover slips as described in the legend to Fig. 1 and incubated for 18 hours in sarcoma-conditioned medium supplemented with the indicated concentration of human leukocyte interferon. At the conclusion of the experiment, the images of the phagokinetic tracks were transferred from the inverted microscope (× 4 objective) to the screen of a Setchell-Carson 10M915 television by means of an RCA TC1005 video camera. The images were then traced from the television screen onto sheets of transparent plastic that were subsequently placed on the magnetic tablet of a Zeiss MOP-3 digital image analyzer. The tracings of the phagokinetic tracks were then retraced with the magnetic stylus of the MOP-3 and automatically processed for dimensions of area (shown in figure) as well as maximum diameter and length of perimeter (not shown). Each point represents the mean area for 100 tracks ± standard error of the mean. Inset indicates values for control cells in Dulbecco's modified Eagle medium supplemented with 10 percent calf serum (*DME-10 CS*), in sarcoma-conditioned medium (*Sarc-CM*) alone, or supplemented with 64 units of human leukocyte interferon per milliliter (*Sarc-CM + HLI*), or an equivalent concentration of mock interferon (*Sarc-CM + MI*) or with human leukocyte interferon that had been preincubated for 1 hour with rabbit antiserum to human leukocyte interferon (*Sarc-CM + HLI + AS*).

markedly and reversibly inhibits this tumor-induced cell motility. Spontaneous migration of other cell types is also inhibited by interferon.

Since human leukocyte interferon has high antiviral activity when tested in bovine cells (*5*), we tested a preparation of human leukocyte interferon (*6*) for its ability to inhibit cell motility, using the clonal line of bovine capillary endothelial cells recently isolated by Folkman *et al.* (*7*). These cells migrate slowly in normal growth medium containing 10 percent calf serum but move rapidly in medium conditioned by previous incubation with cultured cells of mouse sarcoma 180 (tumor-conditioned medium). Addition of human leukocyte interferon to tumor-stimulated bovine capillary endothelial (BCE) cells markedly inhibited cell motility as reflected in the reduced size of the phagokinetic tracks (Fig. 1). The inhibition was dose-dependent, complete inhibition being observed with as few as 6.4 antiviral units (Fig. 2) (1 unit is equal to the amount of interferon needed to inhibit viral multiplication by 50 percent). Motility is expressed in units of area (in square micrometers) of the phagokinetic tracks (*8*).

To confirm that the observed inhibition of cell motility was indeed due to the action of interferon, the cells were incubated with control preparations (mock interferon) prepared from cells according to the same method that was used for production of interferon but without inducer. These preparations had no inhibitory activity on BCE cell motility when tested at the same protein concentrations at which the interferon preparations were active (Fig. 2, inset). In addition, treatment with antiserum against human leukocyte interferon (*9*) totally abolished the inhibitory activity of the interferon preparation (Fig. 2, inset). The motility-inhibitory activity was neutralized by the antiserum to the same extent as was the antiviral activity. When tested by itself, the antiserum had no positive or negative effect on BCE cell motility (data not shown).

Since interferon inhibits both proliferation and migration of BCE cells (*10*), it was important to demonstrate that these effects were reversible and not the result of toxicity. Therefore, cultures were incubated with tumor-conditioned medium plus interferon for 18 hours, then washed twice with growth medium, and incubated for an additional 18 hours in the presence of tumor-conditioned medium without interferon. The mean area of the phagokinetic tracks of these cells was 86 percent greater than control (unstimulated) values. Values for these cultures are nearly identical to those for cultures that were plated directly into interferon-free, tumor-conditioned medium and incubated for 18 hours, then washed twice and incubated for an additional 18 hours in tumor-conditioned medium plus interferon (90 percent over control). This experiment indicates that treatment with interferon for 18 hours is not toxic to the cell's ability to recover its migratory capacity after removal of the interferon.

The ability of human leukocyte interferon to inhibit cell motility is not limited to the tumor-stimulated migration of capillary endothelial cells. It is equally effective in inhibiting the spontaneous migration of aortic endothelial cells and of human diploid skin fibroblasts. In addition, the motility-inhibitory activity of interferon isolated from a given source correlates with its antiviral activity for any given cell type tested. For example, the antiviral titer of a preparation of human fibroblast interferon (*11*) was 320,000 when assayed on human skin fibroblasts as compared to 320 when assayed on BCE cells (*12*). Correspondingly, this interferon preparation inhibited BCE cell motility at a maximal dilution of 1:320 whereas human fibroblast motility was inhibited at a dilution of 1:320,000. In a similar experiment, interferon isolated from mouse C243 cells (*13*) had strong antiviral activity (titer, 640,000) in mouse 3T3 cells and it also inhibited motility of these cells. The same interferon had no antiviral activity in either BCE cells or human diploid fibroblasts and had no effect on the motility of either cell type. Our results indicate, therefore, that the ability of interferon to inhibit cell motility is not limited to any one particular cell type and that preparations of interferon from various species and cell types differ with regard to antiviral and motility-inhibitory activity, depending on the target cell tested.

These findings broaden our knowledge

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.

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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.
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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.

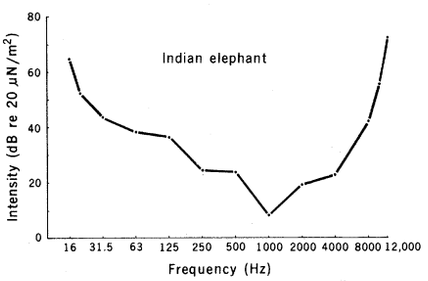

Fig. 1. Audiogram of the elephant. The thresholds represent the average of two audiograms, one obtained with the loudspeaker on the animal's left and the other with the loudspeaker on the right. Frequencies were tested in octave steps from 16 Hz to 8 kHz. Additional frequencies were 20 Hz, 10 kHz and 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

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