low this law. In general, however, the first-order rate equation is inappropriate in this case.

It has been suggested (*2*) that, because of long residence times, the only major sink for F-11 and F-12 is the stratosphere and that CH_3CCl_3 reacts with the HO radical. It is possible that details in the growth curves of Fig. 1 can be explained in this way.

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Whether one uses an exponential model or a linear model to describe the concentration data would matter only if these equations were to be used to predict concentrations of trace gases expected in the future. Such an extrapolation, however, is neither recommended nor was it even implied in (*1*). The rates of increase reported are designed to be used to test or establish the parameters of a global mass balance model and then to compute concentrations expected in the future based on probable scenarios of future global emissions. In general, such projections based on the mass balance of CCl_3F (F-11), CCl_2F_2 (F-12), and CH_3CCl_3 would not agree with either a simple linear or an exponential extrapolation of currently observed increases in these gases. For our data, the exponential model of increase is preferable as we will show here.

Since 1974 the global emissions of CH_3CCl_3 have been rising at about 7 percent per year, and the emissions of F-11 and F-12 may have been falling at 3 to 4 percent per year, although some believe that the annual emissions of F-12 are still rising (*2*). For more than 20 years before 1974, the global emissions of F-11, F-12, and CH_3CCl_3 had been rising exponentially at between 12 and 16 percent per year (*3*). Because of these year-to-year increases, decreases, or fluctuations in the global emissions and the finite lifetimes of these trace gases, matching the observed increase in atmospheric concentrations to a linear function [$c \simeq k_1 t + k_2$, where c is the atmospheric concentration in parts per trillion by volume (pptv), t is the elapsed time, and k_1 and k_2 are constants] is only an approximation, which at least over short time periods is no better than matching

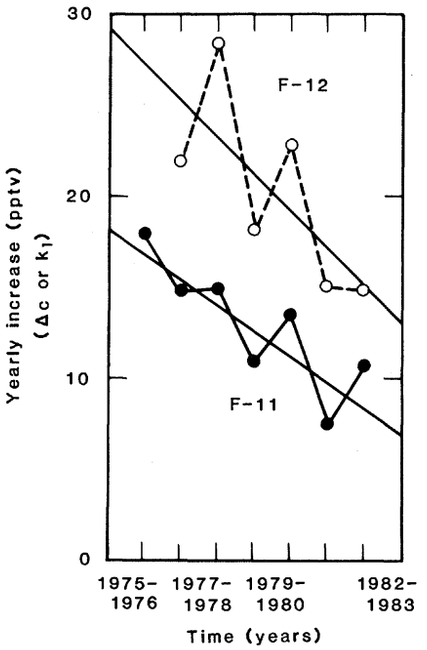

Fig. 1. Decline in the yearly increase in the atmospheric concentrations of F-11 and F-12 over 7 years (1975 to 1982). Linear least-squares calculations are shown by the solid lines.

the atmospheric concentrations to an exponential function ($c \simeq \alpha \exp \beta t \simeq \alpha + \alpha\beta t$). The two functions are practically indistinguishable for many years of data; both can be justified as formal Taylor series expansions, and statistically for our data the linear expression is no better than the exponential one. The reason we chose $\beta[=(1/c)dc/dt]$ is that it expresses a rate of increase that does not require knowledge of the absolute concentration of a trace gas. For gases such as F-11, F-12, N_2O, CH_3CCl_3, and CCl_4, there is still some disagreement on absolute atmospheric concentrations (*4*). If two observers disagree on the absolute concentrations of a trace gas, they will also disagree on the linear rate of increase, but they will agree on the value of β.

The second point Mueller and Kretz make is that the decline in β is due to adding the same increment (Δc) every year to a rising atmospheric burden and not due to a slowdown in the rate of emissions. Thus, according to their linear model, $dc/dt = k_1$ or a constant. We have plotted the measured value of $\Delta c/\Delta t$ from 1975 to 1982 for F-11 and F-12 in Fig. 1. This plot shows that dc/dt is not a constant as Mueller and Kretz claim, and the accumulation rates are declining as we conjectured in (*1*). A linear least-squares analysis of dc/dt shows that $dc/dt = a' + b't$, where $b' < 0$ ($\alpha \leq 0.05$; t-test); $b' = -1.4$ pptv/year^2 for F-11 and $b' = -2$ pptv/year^2 for F-12. It does not matter whether one considers β or k_1; both indicate that the accumulation rates of F-11 and F-12 are slowing down. The case for CH_3CCl_3 is further complicated by cyclic variations in global emissions and its shorter lifetime (6 to 9 years). The case for CH_3CCl_3 is treated in (*5*).

The data on F-11, F-12, and CH_3CCl_3 (*1*) are in accord with current estimates of the global sources and sinks, including globally averaged first-order loss processes due to reactions with tropospheric OH radicals (CH_3CCl_3) or photodissociation in the stratosphere (F-11 and F-12).

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Naloxone and Ischemic Neurologic Deficits in the Gerbil: Is There an Effect?

Hosobuchi and colleagues recently reported in *Science* and elsewhere (*1*) that the opiate antagonist naloxone reversed the ischemic neurologic deficits induced by unilateral carotid ligation in gerbils. Using similar models, we have found that naloxone has no therapeutic effect in experimental stroke in the gerbil (*2*).

From the description given by Hosobuchi *et al.* of the procedures and statistical techniques used (*1*) it appears that the 15 control gerbils that received saline were studied separately from the 10 gerbils that received naloxone and that the raters were not blind as to drug treatments. The degree or lateralization of

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neurologic impairment was not quantified, only 34 of the 59 gerbils with signs of stroke were experimentally accounted for, and a statistical evaluation of the results was not provided.

Because raters using neurologic signs to evaluate function tend to be subjective, it is essential for them to be naïve as to treatment group and for the experimental and control animals to be evaluated simultaneously to eliminate temporal variabilities. We found that simple handling of neurologically impaired gerbils resulted in a decrease in the apparent intensity of signs within 3 to 5 minutes (*2*). This effect, which lasted 15 to 30 minutes (*2*), is therefore similar to what Hosobuchi and colleagues attribute to naloxone treatment in ten gerbils (*1*). For this reason, our treatment groups were run concurrently and neurologic function was evaluated by a rater who was unaware of the treatment categories (*2*).

The pharmacological effects of opiates closely resemble the neurologic deficits induced in gerbils by carotid occlusion (effects on locomotion, decreased responsiveness or "coma," loss of righting reflexes). Indeed, we confirmed that morphine increased the severity of "neurologic" signs as well as mortality. However, demonstration of the stereospecificity of pharmacological responses to injected alkaloid opiates does not prove that *endogenous* opiates acting at opiate receptors are involved in the neurologic deficits produced by permanent unilateral carotid occlusion.

Since we found that naloxone and thyrotropin releasing hormone (TRH) prevent neurologic deficits in cats subjected to experimental spinal cord trauma (*3*), we tested the effects of these drugs on the ischemic neurologic deficits produced by temporary bilateral carotid occlusion in gerbils. Permanent carotid occlusion would have prevented vascular access of injected drugs and blocked any improvement in blood flow to the ischemic areas in question. Unlike temporary carotid occlusion, the permanent unilateral occlusion model appears to be more relevant to problems associated with infarction and cerebral edema (*4*).

Temporary bilateral occlusion appears to result in hypotension as well as neurologic deficits (*2*). The hypotension that followed 15 minutes of bilateral occlusion was reversed by naloxone (5 mg/kg) as well as by TRH (2 mg/kg) injected intravenously.

We evaluated functional neurologic recovery in gerbils that had been subjected to 30 minutes of bilateral carotid occlusion (*5*). Except that TRH increased the respiration rates of these animals, neither naloxone nor TRH had significant effects on time to wake up, time to death, neurologic scores (ptosis, movement, retracted paws, circling, righting reflexes, seizures, and opisthotonus), hot-plate latencies, or roto-rod performance. With ten gerbils per group (*5*), 60 percent of the gerbils were dead within 8 hours (80 percent dead within 3 days) regardless of drug treatment. Thus, despite improved cardiovascular and respiratory function after naloxone or TRH administration, the complete absence of improved neurologic function or survival (*2*) indicated that these drugs were without therapeutic effects in this model of cerebral ischemia (*6*).

Hosobuchi *et al.* (*7*) reported successful reversal of hemiparesis by naloxone in gerbils subjected to permanent unilateral carotid occlusion. Since our results were obtained with temporary bilateral occlusion, we investigated the effects of saline, naloxone, and TRH (as well as morphine) in gerbils subjected to permanent unilateral occlusion. In lightly anesthetized gerbils, the right common carotid was doubly ligated, and the animals were randomly divided into groups that received saline ($N = 27$) or naloxone ($N = 28$) (*5*). Naloxone did not improve the neurologic scores (~ 36 percent had positive signs) or the mortality (~ 25 percent in both groups), respiratory rates, or any other index of neurologic function (*2*). We also attempted to duplicate the methodology of Hosobuchi *et al.*, administering 1 mg of naloxone per kilogram 5 to 6 hours after occlusion, but again we obtained negative results. However, both morphine (10 mg/kg plus one 75-mg pellet) and TRH (as before) exacerbated the neurologic deficits and increased the mortality; 70 percent of the gerbils treated with these drugs showed severe neurologic impairment and died within 7 days (*2*).

Although these authors correctly noted our finding (*3*) that naloxone prevents neurologic deficits (associated with injury-induced neuronal ischemia) following spinal trauma, they were incorrect in reporting that we injected naloxone intracerebrally (or intraperitoneally) in this model. We have no direct evidence that naloxone improves therapeutic outcome centrally since we administered naloxone only intravenously. However, in other studies we have shown central, stereospecific effects of naloxone on blood pressure and respiratory rate in spinal shock produced by complete transection of the cervical cord (*8*). This is a model of neurogenic shock in which cord transection obviously precludes functional neurologic recovery.

The clinical findings by Hosobuchi *et al.* document a lack of effect of naloxone in reversing hemiplegia in one patient with radiographically documented areas of cerebral infarction (*1*). However, naloxone was reported to temporarily reverse the hemiplegia in two patients whose neurologic impairment was not due to hypoperfusion resulting from a permanent infarction. Since naloxone did not work in a patient with documented cerebral infarction, the permanent carotid infarction model in the gerbil appears to have been inappropriate for evaluation of this hypothesis. On the basis of their data with humans (*1*), one would not expect naloxone to improve neurologic deficits induced by permanent carotid occlusion in the gerbil.

Thus Hosobuchi *et al.* do not provide convincing evidence that endorphins are involved in the neurologic deficits caused by permanent unilateral occlusion in gerbils. We found that naloxone had no therapeutic effect on cerebral ischemia in the gerbil. However, by means of an air embolism model of cerebral ischemia in dogs, naloxone was recently shown to improve local cerebral blood flow and somatosensory responses (*9*). Since cerebral ischemia can have many causes that may be of central or peripheral origin, and since different species may vary considerably in their physiological response to trauma [for example, "no reflow" responses (*10*)], it is evident that the fundamental hypothesis that endorphins contribute to the neurologic deficits resulting from cerebral ischemia requires further animal evaluation before human trials are considered.

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5. Three treatment groups received (i) intraperitoneal saline plus a subcutaneous placebo pellet, (ii) naloxone (10 mg/kg; Endo Labs.) plus a 10-

mg naloxone pellet (6), or (iii) TRH (8 mg/kg; Beckman) plus 2 mg of TRH in gelatin. Intraperitoneal drugs (0.10 to 0.12 ml, depending on gerbil size) were injected upon release of the clips, and repository doses were administered (in subcutaneous pellet or gel form) immediately thereafter. Naloxone pellets containing 10 mg of naloxone (free base) were from the same source as those used by Hosobuchi *et al.* We thank H. H. Loh for providing these pellets. The pellets were placed subcutaneously over the hip; the TRH-gel (2 mg of TRH in 0.10 ml) was injected subcutaneously in the same area.

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10. For example, delayed post-ischemic hypoperfusion (no reflow) may be a complication of human cerebral ischemia. No reflow is an infrequent occurrence in gerbil models of temporary carotid occlusion, thus the gerbil model is poor choice for investigations of this phenomenon [D. E. Levy, J. B. Briarley, F. Plum, *J. Neurol. Neurosurg. Psychiatry* **38**, 1197 (1975); K. Nakai, K. M. A. Welch, J. S. Meyer, *ibid.* **40**, 595 (1977)]. See also G. F. Molinari and J. P. Laurent, *Stroke* **7**, 14 (1976); A. G. Waltz, *ibid.* **10**, 211 (1979).

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The role of endogenous opioid ligands in the pathophysiology of cerebral ischemia is still poorly defined, and we enthusiastically support further experiments before naloxone is used widely for the treatment of cerebral ischemia in humans. We cannot agree, however, with the conclusions of Holaday and D'Amato (*1*) concerning our experiments in gerbils.

Holaday and D'Amato (*1*) report that, using two different models of stroke in the gerbil, they could not reproduce our finding (*2*) that naloxone reverses ischemia-induced neurologic deficits in this species. The model they used for most of their experiments—bilateral carotid occlusion to produce a 30-minute "no-flow" state—is a well-accepted model for *global* cerebral ischemia. This model bears no relation to the model of *focal* cerebral ischemia from unilateral carotid occlusion that we used in our experiments. Therefore, the results obtained by Holaday and D'Amato in gerbils that underwent bilateral occlusion are irrelevant to this discussion.

Holaday and D'Amato (*1*) state that only 34 group A gerbils were accounted for; as we reported, of group A gerbils, 15 were used as controls for the mortality study, 9 had naloxone pellets implanted subcutaneously, 10 were tested with intraperitoneal naloxone, and 20 were used for opiate receptor binding studies. The remaining five gerbils in group A were killed and brains were used for analysis of concentrations of immunoreactive β-endorphin-like material.

We were aware that mere handling of experimental animals after unilateral occlusion may arouse them and produce spontaneous improvement in the level of consciousness. For this reason, we used a specific test of neurologic function, the drag test, to evaluate the degree of hemiparesis in each limb of stroked gerbils. Such a forced meter task distinguishes between actual improvement in motor strength and a simple change in arousal state. Holaday and D'Amato used no such test. Instead, they evaluated ptosis, general locomotion, the presence of retraced paws, circling, righting reflexes, opisthotonus, hot-plate latencies, and roto-rod performance. Ptosis reflects damage to the sympathetic nervous system, which is usually secondary to local carotid dissection. Movement, retracted paws, circling, righting reflexes, hot-plate latencies, and roto-rod performance all reflect simple arousal and brainstem function, and are not specific for focal, unilateral higher function. Seizures and opsithotonus do not reflect the degree of focal neurologic deficit in any measurable way. Therefore, the testing carried out by Holaday and D'Amato cannot detect the changes in focal neurologic function that we reported. Moreover, it is not surprising that they did not find a difference between the effects of naloxone and placebo, because they tested for changes in arousal state and brainstem function only. We are aware of the reports that improved survival has been noted with increased sensory stimulation in animals and humans with cerebral dysfunction (*3*), but this arousal phenomenon is not the effect that we have reported.

That Holaday and D'Amato found that a single injection of naloxone did not change the mortality rate of gerbils with stroke secondary to unilateral carotid ligation is even less startling, because the drug has a half-life of approximately 20 minutes in vivo. We reported (*2*) that serial injections of naloxone, which reproducibly reversed hemiplegia in gerbils with stroke, did not change the mortality rate. However, gerbils with subcutaneously implanted naloxone pellets that released the drug *continuously* survived deficit-free for over 2 weeks. Holaday and D'Amato performed no similar experiments.

It is encouraging that Holaday and D'Amato confirmed our finding that morphine exacerbates focal neurologic deficits in symptomatic gerbils with unilateral carotid ligation. What they did not do, however, is test gerbils that were asymptomatic after ligation. As we reported (*2*), these gerbils developed focal deficits after the administration of morphine; the effect was both stereospecific and receptor-specific.

We have recently reported (*4*, *5*) the naloxone reversal of ischemic neurologic deficits in cats and baboons that underwent middle cerebral artery occlusion. In neither animal model did we note changes in blood pressure, heart rate, arterial blood gases, cardiac output, or cardiac filling pressures after naloxone administration despite reversal of neurologic deficit.

Interlaboratory discrepancies in results of experiments with gerbil models are characteristic of research in stroke. The age and sex of gerbils play a significant role in the size and extent of induced infarction and in the response to therapeutic intervention (*6*). Holaday and D'Amato do not specify either the age or sex of the gerbils used in their experiments. We used adult male gerbils, and a different population may give different results.

The results reported by Holaday and D'Amato in no way alter our contention that the effects of opiate agonists and antagonists on focal ischemic neurologic deficit suggests a role for opiate receptors and endogenous opioid peptides in the pathophysiology of stroke. The experiments of Holaday and D'Amato were conducted primarily with a model of global, not focal, ischemia, and omitted specific assessment of higher cortical focal neurologic function. Furthermore, the naloxone was not released continuously in accordance with our methodology and the stereospecific and receptor-specific effects of opiate agonists were not examined.

We do agree, however, that extensive clinical trials of opiate antagonists in patients with cerebral ischemia and infarction could be premature until the mechanism by which this important effect is mediated is more extensively defined.

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