to represent a normal plasma-urine transfer time.

The most likely explanation for the delay is that some stress-related but delayed process occurred in several of our subjects some time after the parachuting experience. Although the process is unknown, the data suggest that stress can increase the amount of circulating PEA.

MANLEY A. PAULOS
Department of Psychology, University of Kansas, Lawrence 66045
RICHARD E. TESSEL
Department of Pharmacology and Toxicology, University of Kansas

References and Notes

1. D. M. Paton, Ed., *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Raven, New York, 1976), p. 155; E. A. Meja, D. W. Stoff, J. C. Gillin, R. J. Wyatt, *Mod. Pharmacol. Toxicol.* **12** (part 1), 315 (1978).
2. S. G. Potkin, F. Karoum, L.-W. Chuang, H. E. Cannon-Spoor, I. Phillips, R. J. Wyatt, *Science* **206**, 470 (1979).
3. J. Zubin and B. Spring, *J. Abnorm. Psychol.* **86**, 103 (1977); E. Roberts, in *Neuroregulators and Psychiatric Disorders*, E. Usdin, D. A. Hamburg, J. D. Barchas, Eds. (Oxford Univ. Press, London, 1977), p. 578; G. Gardos and J. O. Cole, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1978), p. 1169.
4. J. M. Davis and R. F. Schlemmer, Jr., in *Amphetamines and Related Stimulants: Chemical, Biological, Clinical and Sociological Aspects*, J. Caldwell, Ed. (CRC Press, Boca Raton, Fla., 1980), p. 161.
5. H. Utena, in *Biological Mechanisms of Schizophrenia and Schizophrenia-like Psychosis*, H. Mitsuda and T. Fukuda, Eds. (Igaku Shoin, Tokyo, 1974), p. 285.
6. Urine samples were collected and immediately frozen (−20°C) for transport to long-term storage (−70°C). In the analysis, 1-ml portions of each sample were mixed with 0.5 ml of 0.5*M* phosphate buffer. An internal standard (deuterated PEA) was added and the mixture was extracted into ether, then back-extracted into 0.1*N* HCl, which was blown off with argon. The PEA in the residue was derivatized with 5 percent pentafluoropropionyl imidazole in ethyl acetate (200 μl) by heating at 80°C for 10 minutes. The ethyl acetate and excess derivatizing agent were blown off with argon. The derivatized PEA was reconstituted with 50 μl of ethyl acetate. Gas chromatography was carried out on a 30 m by 0.32 mm (inside diameter) fused-silica capillary column. A quadrupole gas chromatograph–mass spectrometer (Ribermag R10-10) focused on ions of 104 and 107 amu to detect PEA and [$^2H_4$]PEA, respectively (*12*).
7. Heart rate and electrocardiogram (EKG) were recorded with a Holter monitor (Dynagram 5000 portable electrocardiograph), which was attached to each subject for continuous recording. The monitor was attached (three leads, modified V_4-V_5 recording) 1 hour before the jump, remained attached during the jump, and was removed 1 hour after the jump for a total of 2 hours of recordings. The EKG was recorded on cassette tape for later analysis by J. Schroeder, Department of Cardiology, Stanford University Medical Center. One hour before the jump, mean heart rate was 83 ± 5 beats per minute. Ten minutes after the jump, heart rate was maximally elevated (141 ± 7 beats per minute). One hour after the jump, heart rate had returned to 83 ± 5 beats per minute. No clear EKG abnormalities were noted.
8. K. B. Houston, *J. Pers.* **41**, 50 (1973).
9. A. D. Mosnaim and M. E. Wolf, *Mod. Pharmacol. Toxicol.* **12** (part 1), 3 (1978); J.-S. Kim, H. Uchimura, M. Hirano, M. Saito, T. Matsumoto, T. Nakahura, *Biochem. Pharmacol.* **30**, 893 (1981).
10. Mean urinary *p*H for all subjects (N = 75) was 5.83 ± 0.07; urinary PEA excretion was not correlated with urinary *p*H.
11. V. M. Petrović and V.-J. Sibalić, in *Catecholamines and Stress: Recent Advances*, E. Usdin, R. Kvetnansky, I. J. Kopin, Eds. (Elsevier, New York, 1980), p. 365.
12. F. Karoum, H. Nasrallah, S. Potkin, L. Chuang, J. Moyer-Schwing, I. Phillips, R. J. Wyatt, *J. Neurochem.* **33**, 201 (1979).
13. R. J. Wyatt, S. G. Potkin, J. C. Gillin, D. L. Murphy, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1978), p. 1083.
14. J. Giogio, in *Clinical Chemistry: Principles and Techniques*, R. J. Henry, D. C. Cannon, J. W. Winkelman, Eds. (Harper & Row, Hagerstown, Md., 1974), p. 541.
15. W. R. Faulkner and J. W. King, in *Fundamentals of Clinical Chemistry*, N. W. Tietz, Ed. (Saunders, Philadelphia, 1976), p. 975.
16. T. B. Vree and J. M. van Rossum, in *Amphetamine and Related Compounds*, E. Costa and S. Garattini, Eds. (Raven, New York, 1970), p. 165.
17. We thank C. Judson and J. Storey for technical assistance. Supported in part by PHS grants DA-01614, GM-27430, RR-05606 and by a grant from the University of Kansas General Research Fund. Submitted by M.A.P. in partial fulfillment of the requirements for the Ph.D. degree in psychology at the University of Kansas.

8 December 1981

Mass Mortality of Harbor Seals: Pneumonia Associated with Influenza A Virus

Abstract. *More than 400 harbor seals, most of them immature, died along the New England coast between December 1979 and October 1980 of acute pneumonia associated with influenza virus, A/Seal/Mass/1/80 (H7N7). The virus has avian characteristics, replicates principally in mammals, and causes mild respiratory disease in experimentally infected seals. Concurrent infection with a previously undescribed mycoplasma or adverse environmental conditions may have triggered the epizootic. The similarities between this epizootic and other seal mortalities in the past suggest that these events may be linked by common biological and environmental factors.*

There have been three known incidents of unexplained large-scale mortalities which we think may be attributed to pneumonia in seals (*1–3*). While monitoring marine mammal strandings along the New England coast, we studied such an event in harbor seals (*Phoca vitulina*) that began in December 1979. The seals died of an acute and devastating pneumonia that we associate with a previously undescribed influenza virus and a mycoplasma. We suggest that the influenza virus, prompted by environmental conditions and the presence of the mycoplasma, may have been responsible for similar occurrences in the past (*1–3*).

The disease first appeared within a tight grouping of seals on Billingsgate Shoal, Cape Cod. It spread rapidly, killing at least 130 animals within a month. Thereafter, the mortality rate declined as the seals dispersed northward along the New England coast (Fig. 1). By October 1980, when the outbreak had run its course, at least 445 harbor seals had died. Ninety percent of the dead seals were under 3 years of age; males and females were equally affected.

The clinical signs of the disease were dramatic. Obviously well-nourished seals appeared weak, moving feebly and without coordination, and exhibited respiratory distress. Occasionally, one would thrash its head to clear a frothy white or bloody discharge from the airways. Otherwise the seals remained still, except for quivering of their muscles. Characteristically, their necks were swollen because of entrapped air that escaped from the lungs, through the thoracic inlet, and into the muscles and fascia of the neck and back. This caused some seals to be so buoyant that they drifted with the wind and tide. We estimate that the disease took 3 days or less to develop. The most acutely affected seals died within hours after feeding normally.

On postmortem examination of animals that died of the disease, we found pneumonia characterized by necrotizing bronchitis and bronchiolitis, and hemorrhagic alveolitis. We isolated an assortment of aerobic and anaerobic bacteria from the lungs (*4*) but could not implicate any as the cause of the disease. However, a mycoplasma was isolated from the lungs of all of the eight animals from which culture was attempted. The organism hydrolyzed arginine; it was also nonglycolytic, sensitive to digitonin, and resistant to penicillin, penicillin derivatives, and erythromycin. Thus far, the organism has been tested with antiserums to 55 known species and strains of mycoplasma and has shown no fluorescent antibody staining reaction or growth inhibition. Although mycoplasmas are not normally considered to be primary agents of such severe and widespread disease, we are nevertheless investigating the pathogenicity of this unidentified organism in seals.

The pathologic findings and the natural history of the epizootic pointed to a viral etiology. This assumption was strengthened by the isolation, in three independent laboratories, of an influenza A virus (A/Seal/Mass/1/80) from the lungs and brains of the dead seals (*5*,

0036-8075/82/0226-1129$01.00/0

6). The virus was antigenically similar to A/Fowl plague/Dutch/27 (Hav1Neq1) (H7N7), with all genome segments closely related to those from avian viruses. However, the virus replicated better in mammalian hosts (pigs, ferrets, cats, and seals) than in avian species (chicken, turkeys, ducks). It caused conjunctivitis in an accidentally infected man (7), and mild respiratory disease in experimentally inoculated seals (6).

Considering that the virus may have played a prime role in the Cape Cod outbreak, we sought to identify environmental or biological factors that normally act in conjunction with influenza viruses to produce disease (8). Lung parasites have been correlated with severe influenza attacks in swine (9). However, the prevalence of lungworm (*Otostrongylus circumlitus*) and heartworm (*Dipetalonema spirocauda*) in affected seals (21 and 63 percent, respectively) did not differ from that which we have observed previously in over 200 stranded harbor seals. We also ruled out bacteria as predisposing factors, since no single bacterium was consistently isolated from infected animals (4). Mycoplasma was so isolated, however, and we are now studying the effect of combined virus and mycoplasma infections in seals.

We believe that population density and environmental factors may have promoted the epizootic. At the time of the Cape Cod outbreak there were unusually large numbers of seals gathered on Billingsgate Shoal (10), reflecting the doubling of the New England harbor seal population since 1972 (11). In addition, there were unseasonably warm temperatures (12) which can induce seals to spend more time ashore (13). Similar conditions prevailed in the Antarctic in 1955 when 85 percent of a local population of 2500 young crabeater seals (*Lobodon carcinophagus*) died of pneumonia attributed to a "virus infection" (2). As in the 1980 epizootic, the crabeater seal population was considerably larger than that normally wintering in the area, and the climate was unusually mild. There have been two other reports of outbreaks of a pneumonia-like disease in seals (1, 3) but neither report provides details of associated biological and environmental conditions.

We do not know how and when the A/Seal/Mass/1/80 virus was transmitted to the harbor seals. Characteristics of the virus suggest that it is of avian origin, and may have been introduced by birds. Terns (*Sterna* sp.), for example, are known to harbor influenza A viruses and to associate with seals in water and on land. Transmission need not have been direct or recent; other marine organisms, including mammals, may have carried the virus. Indeed there is evidence of influenza viruses in two marine mammal species from the North Pacific (14, 15) and there are two accounts of influenza-like epizootics in widely separated harbor and crabeater seal populations (2, 3). In each case the clinical signs resembled those observed in the Cape Cod harbor seals and included, in the crabeater seals, swollen necks.

If these events are associated with influenza, they may be linked by a common virus transported by a highly mobile carrier, or they may be the result of unrelated viruses latent within each population. In searching for evidence of influenza viruses in a broad range of marine organisms we found that antibodies to A/Seal/Mass/1/80 virus are present in gray seals (*Halichoerus grypus*) on Sable Island, Nova Scotia, 400 miles from the closest known infected harbor seal and 500 to 600 miles from the center of the outbreak. Although this finding provides evidence that the virus can replicate in another seal species, it does not explain the mechanism by which such viruses may be transferred to distant populations.

In studying this epizootic we attempted to determine its impact on the New England harbor seal population. During the first 2 months of the outbreak mortality was high, approaching 25 percent of the estimated 600 seals on Billingsgate Shoal (10). As the seals dispersed in the spring the death rate declined abruptly. Ultimately, at least 445 seals, representing 3 to 5 percent of the 10,000 to 14,000 along the New England coast (11), died of the disease. Probably more seals that were not recovered, died, and still others may have suffered long-term effects, including lung damage that impaired their capacity to dive and feed. Reproduction may also have been affected; the only pregnant seal observed with the disease aborted soon after it was brought into captivity. Nevertheless, since October 1980, when we last isolated the virus from an infected seal, mortality as judged by the number of dead seals found ashore has remained stable at pre-epizootic levels, suggesting that the epizootic was a transient event, having had a conspicuous impact on a small group within the population.

The 1980 epizootic is significant in

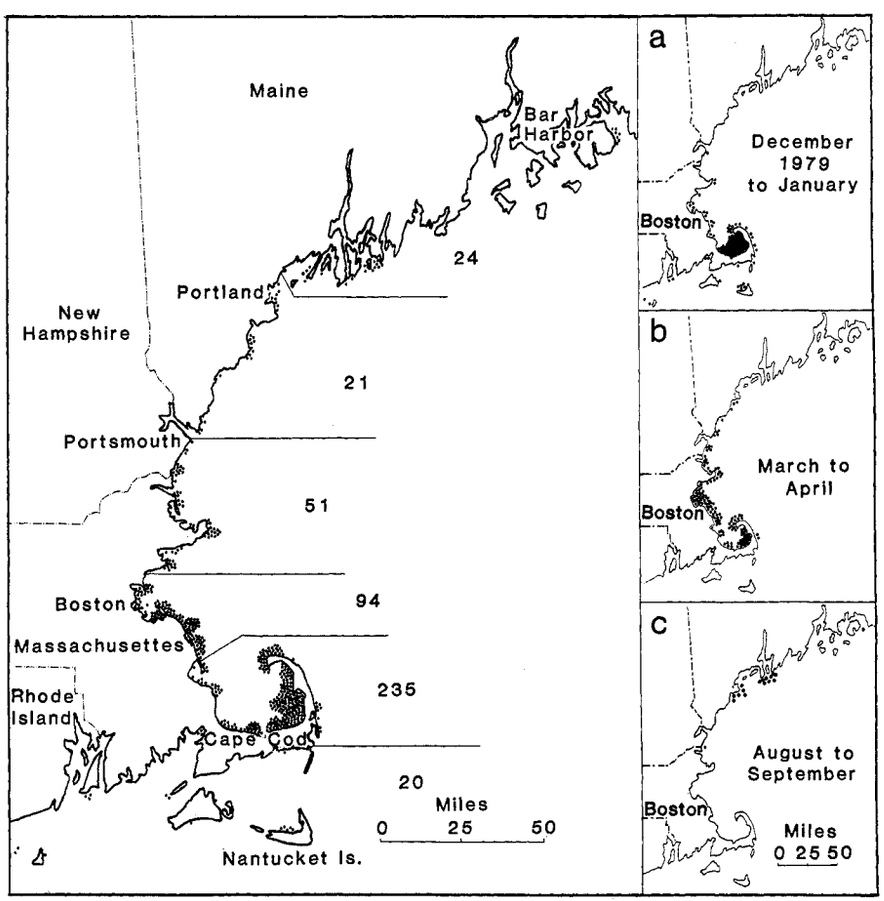

Fig. 1. Regional distribution of 445 harbor seal carcasses recovered from December 1979 to November 1980. The outbreak began in Cape Cod Bay (a), and moved northward through the spring and summer (b and c). Each dot represents a seal.

several respects. It is the first evidence that an influenza virus antigenically and genetically related to avian viruses can be associated with severe disease in wild mammals. As with influenza in swine, concurrent respiratory infection with an organism such as mycoplasma and adverse environmental conditions may have triggered the expression of a latent virus. The striking similarities between the harbor seal epizootic and large-scale seal mortalities in the past suggest that these events may be linked by common biological and environmental factors that we are only beginning to recognize.

J. R. GERACI
D. J. ST. AUBIN
I. K. BARKER
Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1
R. G. WEBSTER
V. S. HINSHAW
W. J. BEAN
St. Jude Children's Research Hospital, Memphis, Tennessee 38101
H. L. RUHNKE
Veterinary Services Branch, Ontario Ministry of Agriculture and Food, Guelph N1H 6R8
J. H. PRESCOTT
G. EARLY
New England Aquarium, Boston, Massachusetts 02110
A. S. BAKER
S. MADOFF
R. T. SCHOOLEY
Massachusetts General Hospital, Boston 02114

References and Notes

1. J. B. Gallagher and W. E. Waters, *J. Zool.* **142**, 177 (1964).
2. R. M. Laws and R. J. Taylor, *Proc. Zool. Soc. London* **129**, 315 (1957).
3. G. G. Bardarson, *Natturufraedingurinn* **1**, 27 (1931).
4. Bacterial isolates included α and β streptococci, *Escherichia coli, Staphylococcus epidermidis*, and *S. aureus*; *Clostridium* species and *C. perfringens*; *Neisseria, Klebsiella, Pseudemonas*, and *Pasteurella* species; and *Actinobacillus actinomycetemcomitans* and *Bordetella bronchiseptica*. The isolates were identified by the Infectious Disease Unit, Massachusetts General Hospital, Boston, and J. Prescott, Department of Veterinary Microbiology and Immunology, University of Guelph.
5. G. Lang, A. Gagnon, J. R. Geraci, *Arch. Virol.* **68**, 189 (1981).
6. R. G. Webster *et al.*, *Virology* **113**, 712 (1981).
7. R. G. Webster, J. R. Geraci, G. Petursson, K. Skirnisson, *N. Engl. J. Med.* **304**, 911 (1981).
8. B. C. Easterday, in *The Influenza Viruses and Influenza*, E. D. Kilbourne, Ed. (Academic Press, New York, 1975), pp. 449–481.
9. D. Nayak, G. Kelley, N. Underdahl, *Cornell Vet.* **54**, 160 (1964).
10. S. Kraus, New England Aquarium, unpublished observation.
11. J. R. Gilbert, University of Maine, personal communication.
12. The recorded monthly averages for December 1979 and January 1980 at Plymouth, Cape Cod, were 1.1°C above the 50-year mean of 0.6°C. National Oceanic and Atmospheric Administration, Environmental Data Service.
13. J. Boulva and I. McLaren, *Bull. Fish. Res. Board Can.* **200**, 1 (1979).
14. D. K. Lvov *et al.*, *Bull. WHO* **56** (No. 6), 923 (1978).
15. A. A. Sazonov *et al.*, in *Ecology of Viruses*, D. K. Lvov, Ed. (USSR Academy of Medical Sciences, Ivanovsky Institute of Virology, Moscow, 1976), Issue 4, pp. 157–160; *ibid.*, Issue 3, pp. 150–154.
16. Field officers of the U.S. National Marine Fisheries Service (NMFS), U.S. National Park Service, U.S. Department of Agriculture, Massachusetts Department of Natural Resources, Maine State Department of Marine Research, Canadian Fisheries and Oceans (CFO), Sealand of Cape Cod, and the International Fund for Animal Welfare coordinated the collection of seals. R. Prescott, Cape Cod Museum of Natural History, and C. Skinder, New England Aquarium, gave technical assistance. M. Rosenfeld, Manomet Bird Observatory (Massachusetts), provided field descriptions. R. A. Del Guidice, Frederick Cancer Research Center, Frederick, Md., and J. G. Tully, National Institutes of Health, Bethesda, Md., helped to characterize the mycoplasma. S. Marsh and C. Rodd prepared the figure and M. Hundleby critically reviewed the manuscript. This study was supported by grants AI02649 and AI16841 from the National Institute of Allergy and Infectious Diseases, by the NMFS, CFO (01SUFP802-0-2186), Arco Foundation, and by Natural Sciences and Engineering Research Council of Canada (A6130).

13 October 1981

Computer-Detected Patterns of Electroencephalographic Delta Activity During and After Extended Sleep

Abstract. *Delta (0.5 to 3 hertz) waves are the electroencephalographic hallmark of human sleep. We measured their rate of production during and following an extended night of sleep. On the extended night, we confirmed previous observations of a linear decline in delta wave production across the first four periods of non-rapid-eye-movement (non-REM) sleep. An asymptote was reached in the fifth non-REM period, perhaps signifying that sleep processes reached completion. On the day after the extended night, subjects were allowed to remain awake 3.6 hours less than normal. During the next sleep session, amplitude and number of delta waves in non-REM periods 1 and 3 were significantly reduced. These findings illustrate the value of computer analysis of electroencephalographic waveforms in sleep. Systematic measurement of the amount and distribution of these waveforms as a function of preceding waking duration should provide clues to the kinetics of the metabolic processes underlying sleep.*

Stage 4 sleep, characterized by dense, high-voltage delta (0.5 to 3 Hz) electroencephalographic (EEG) waves (*1, 2*) is of interest because (i) the amount depends on the duration of waking that has preceded sleep (*3*); (ii) delta sleep diminishes steeply across the night (*1, 4*) as though some substrate were being consumed (*5, 6*); (iii) stage 4 sleep occurring in daytime naps reduces the amount of stage 4 in the subsequent night's sleep, whereas rapid-eye-movement (REM) sleep during naps does not affect subsequent REM (*5*); (iv) delta sleep remains at normal levels or higher when total sleep time is restricted for long periods, whereas REM sleep is sacrificed (*7*); and (v) stage 4 is the sleep stage most strongly affected by age over the human lifespan (*6, 8*). These facts led us to suggest that delta waves are an electrophysiological correlate of those unknown metabolic processes by which sleep reverses the effects of waking on the brain (*6*).

Despite the intensive research on sleep during the past 25 years, and despite growing recognition of the importance of delta sleep, some of its basic characteristics have not yet been fully established. We do not know whether a longer period of wakefulness increases stage 4 sleep by increasing the density, duration, or amplitude of delta waves, each of which affects the visual scoring of this stage (*2, 9*). The effects of variations in duration of wakefulness on the distribution of delta waves across the night's sleep are also undetermined. These questions can now be investigated effectively with relatively simple computer techniques (*10*). We applied such methods in a study of EEG patterns during and after extended sleep. The visually scored data have been reported (*11*). Here, we present new observations on the trends in delta production across a night of extended sleep and on the effects of a reduced time awake on the number, amplitude, and distribution of delta waves during subsequent non-REM sleep.

Eye movements and EEG were recorded in 19 medical and 2 undergraduate male students (median age, 24.1 years; range 20.3 to 29.3 years) on three consecutive nights. The subjects were in bed for 8 hours on the adaptation night, 12 hours on the extended night, and 8 hours on the recovery night, beginning at 11 p.m. each night (*12*). They averaged 428, 641, and 427 minutes of sleep, respectively, on the three nights (*13*). Thus, the duration of waking in the 24-hour period preceding the extended night was 1012 minutes and that in the 24 hours preceding the recovery night, 799 minutes.

Table 1 gives durations of non-REM

0036-8075/82/0226-1131$01.00/0