preserved. No ova or parasites were seen.

No traces of histologic structure were seen in the other tissues examined, including the heart and spleen of the rabbit, skin and muscle of the mammoth and lynx, and the horse marrow. No pathologic changes were recognized. The rehydration of the lemming (or vole) was only partially effective and did not aid in its identification.

Our study demonstrates the preservative effect of freezing and subsequent mummification to last much longer than previously suspected. Such results are very encouraging to the paleopathologist, interested in much more recent human remains, and to the paleontologist, for whom this technique may prove useful in studying evolutionary change at the microscopic level. It has been suggested that most human infections originated as zoonoses (*13*). The preservation of normal histologic structures in these ancient animal remains raises the possibility that the demonstration of disease organisms could yield evidence on this thesis.

On the other hand, the type and degree of destruction of tissue indicate that sufficient time elapsed between death of these animals and their entombment in the permafrost zone to allow considerable decay. This finding, plus the rarity of complete mummies of the larger species, demonstrates that after death these mammal remains were usually dismembered and partly decomposed before their entombment by the normal depositional processes of a periglacial environment. These conclusions directly counter the popular notion that the mummified remains indicate rapid freezing under conditions of catastrophic climatic change (*14*).

MICHAEL R. ZIMMERMAN
Departments of Pathology and *Anthropology, University of Pennsylvania, Philadelphia 19104*
RICHARD H. TEDFORD
Department of Vertebrate Paleontology, American Museum of Natural History, New York 10024

References

1. T. L. Péwé, *U.S. Geol. Surv. Prof. Pap. No. 835* (1975).
2. J. P. Tolmachoff, *Trans. Am. Philos. Soc.* **23**, 11 (1929).
3. W. F. Farrand, *Science* **133**, 729 (1961).
4. T. L. Péwé, N. L. Rivard, G. A. Llano, *ibid.* **130**, 716 (1959).
5. A. V. Sher, *Mammals and Stratigraphy of the Pleistocene of the Extreme Northeast of the U.S.S.R. and North America* (Nauka, Moscow, 1971).
6. T. A. Cockburn, R. Barraco, T. A. Reyman, W. H. Peck, *Science* **187**, 1155 (1975); M. R. Zimmerman, *Paleopathol. Newsl. No. 3* (1973), p. 11; A. T. Sandison, in *Science in Archaeology*, D. Brothwell and E. Higgs, Eds. (Praeger, New York, ed. 2, 1970), pp. 490–502.
7. S. I. Rudenko, *Frozen Tombs of Siberia* (Univ. of California Press, Berkeley, 1970); M. I. Artamonov, *Sci. Am.* **212**, 101 (May 1965).
8. T. Pizzi and H. Schenone, *Bol. Chile Parasitol.* **9**, 71 (1954).
9. M. R. Zimmerman and G. S. Smith, *Bull. N.Y. Acad. Med.* **51**, 828 (1975).
10. H. C. Ezra and S. F. Cook, *Science* **129**, 465 (1959).
11. M. A. Ruffer, *Studies in the Paleopathology of Egypt* (Univ. of Chicago Press, Chicago, 1921).
12. L. Luna, *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology* (McGraw-Hill, New York, 1968).
13. T. A. Cockburn, *Curr. Anthropol.* **12**, 45 (1971); D. Brothwell, in *Science in Archaeology*, D. Brothwell and E. Higgs, Eds. (Praeger, New York, ed. 2, 1970), pp. 310–314.
14. I. T. Sanderson, *Saturday Evening Post* (16 Jan. 1960), p. 39.

8 April 1976; revised 28 June 1976

Bat Mortality: Pesticide Poisoning and Migratory Stress

Abstract. *Organochlorine residues in the fat of young Mexican free-tailed bats,* Tadarida brasiliensis, *reached the brain and caused symptoms of poisoning after the fat mobilization that takes place during migratory flight was simulated. These chemical body burdens were obtained naturally under free-living conditions at the maternity roost. The data obtained support the hypothesis that pesticides have contributed to recent declines in populations of this bat.*

The Mexican free-tailed bat, *Tadarida brasiliensis*, is a migratory, colonial species. Each spring millions of individuals migrate north from wintering areas in Mexico to maternity roosts in the southwestern United States (*1*). In the late 1950's and early 1960's, about 150 million free-tailed bats were estimated to be living in 20 maternity colonies (*2*). It is estimated that, before the southward migration in October, such a bat population would consume more than 18,000 metric tons of insects (*3*). Recent observations, however, indicate that there have been drastic declines in populations of *T. brasiliensis*. For example, the size of the summer populations at Carlsbad Caverns, New Mexico, declined from an estimated 8.7 million in 1936 (*4*) to 200,000 in 1973 (*5*), and the population at Eagle Creek Cave, Arizona, dropped from about 25 million in 1964 (*1*) to 600,000 in 1970 (*6*). Pesticides and direct human disturbances have been suspected as possible causative agents in these declines (*7*). A recent study of pesticide residues in *T. brasiliensis*, however, showed no cause-and-effect relationship (*8*).

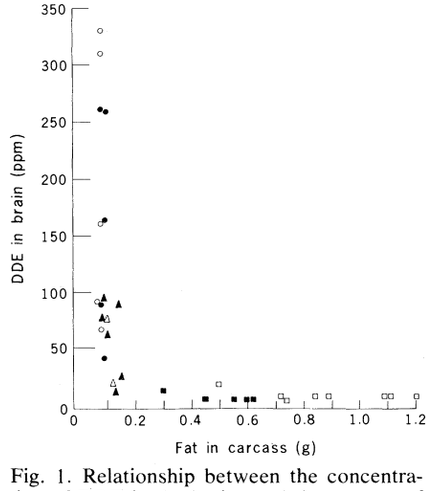

Fig. 1. Relationship between the concentration of DDE in the brains and the amount of fat in the carcasses of *Tadarida brasiliensis* from the reference (squares), unexercised (triangles), and exercised (circles) groups. Open symbols represent younger animals, and closed symbols represent older animals.

Free-tailed bats are born in early summer and reach adult size before leaving on their southward migration. Deposition of fat and a concomitant buildup of pesticide residues in nursing young continue until they begin to fly (*8*, *9*). Since organochlorine pesticides are fat-soluble and are readily stored in fat, individuals may not exhibit toxic effects unless fat reserves are used (*10*). However, rapid mobilization of pesticide-loaded fat can result in significant increases in the amounts of pesticide residues in the brain and can cause death (*11*, *12*). Because the maximum storage of fat and pesticides in *T. brasiliensis* occurs toward the end of nursing, we hypothesized, as others have (*8*), that the critical stage in the life cycle of these bats may be during the initial migratory flight, when the rapid mobilization of fat releases toxic residues that may reach the brain in lethal or detrimental amounts. By simulating in the laboratory the fat mobilization that occurs during migratory flight, we have demonstrated that significant increases in the organochlorine residues can occur in the brains of young, flying *T. brasiliensis*.

On 28 August 1974, 20 young *T. brasiliensis* were selected from bats netted during the evening exit flight from Carlsbad Caverns. Each bat was marked, the sex and body weight were recorded, and the relative age was determined from the amount of cartilage around the finger joints (*13*). The next morning these bats were transported to the University of New Mexico, Albuquerque, where they were housed in Wahmann slant cages (38 by 23 by 18 cm) and kept in an environmental chamber under a photoperiod of

14 hours of light in 24 hours (lights on at 0600; off at 2000). The mean chamber temperature was 29.5°C (range, 28.9° to 30.0°C), and the mean water vapor pressure was 16.4 mm-Hg (range, 14.6 to 18.1 mm-Hg). The bats were not fed but were individually given water from a syringe twice a day, thus reducing weight loss through dehydration. Beginning on their first day in captivity, the bats were induced to fly for 30 minutes each day in a flight chamber (17.1 by 4.9 by 2.7 m). For bats that would not or could not fly, other exercises such as crawling and righting were employed. Ten bats that refused to drink during the first few days were eliminated from this experimental group. The ten remaining animals were used to complete the experiment. At death, each bat was weighed, wrapped in aluminum foil, frozen, and stored at −20°C for later pesticide and fat analysis (*14*).

Another experimental group of ten young *T. brasiliensis* was obtained at Carlsbad Caverns on 7 September 1974. These bats were treated in the same way as the earlier group except that their cages were kept in a vivarium with a mean room temperature of 21.1°C (range, 20.6° to 21.7°C) and a mean water vapor pressure of 8.5 mm-Hg (range, 7.1 to 10.9 mm-Hg). These bats were never allowed to fly nor were they fed, but they were given water twice a day; nondrinkers, as before, were eliminated. The eight individuals that drank were frozen for analysis (*14*) when they died or when killed at the end of the experiment.

A reference group of 13 young *T. brasiliensis*, netted at Carlsbad Caverns on 19 August 1974 and frozen for analysis (*14*) immediately after their sex and relative age had been recorded (*13*), provided an estimate of the pesticide residues and amounts of fat in the population before migration.

The carcasses of older, flying young from the reference group contained significantly less fat than those of younger individuals ($P < .01$, Fig. 1) (*15*). A reduction in the amount of all chemical residues in the carcass accompanied this decrease in carcass fat (the reductions in DDE and DDT were significantly lower, $P < .01$, Table 1) (*15*). Apparently, as the flying young mature in the maternity roost, the mobilization and excretion of pesticides occur concurrently with the mobilization and depletion of body fat. The fat content ($P > .5$) and the amounts of chemical residues ($P > .1$, Table 1) in the brain, however, remain constant during this period.

Exercised young died between day 6 and day 9 after capture. Although the older bats had less body fat than the younger ones at the beginning of the experiment (as shown by the reference group) and survived for a slightly shorter period (means, 7.2 and 8.0 days, respectively), the amount of carcass fat at death was essentially the same in both groups (0.08 and 0.09 g, Fig. 1).

Two of the unexercised young died on day 6 and two on day 8; the remaining four (two each from the younger and older groups) were alive at the end of the experiment on day 9. Bats in the unexercised group survived longer and lost significantly less carcass fat than those in the exercised group ($P < .01$, Fig. 1). The difference in the fat content was clearly a result of the fact that the unexercised animals remained in a torpid condition during the day. Their mean daytime body temperature was 21.9°C, whereas that of the exercised group was 30.5°C (*16*). At night, however, animals of both groups were active in their cages.

As expected, the amount of body fat in carcasses was much lower in experimental groups than in the reference group ($P < .001$, Fig. 1). The amount of fat in the brain, however, did not decrease significantly ($P > .05$). Rapid use of the carcass fat in the experimental animals was accompanied by pronounced increases in the amounts of all chemical residues in the brain (for DDE and DDT, $P < .002$) (Table 1). On the basis of the median concentration of DDE in the brains before fat utilization [determined from the animals in the reference group—3.7 parts per million (ppm) for the younger and 1.3 ppm for the older bats], DDE in the brains of the unexercised bats increased by a factor of 12.7 in the younger individuals and by a factor of 53.8 in the older ones (Fig. 1). In the exercised group, DDE increased by a factor of 43.2 in the younger individuals and by a factor of 123.1 in the older ones (Fig. 1). The concentrations of the other chemicals also increased but never exceeded 1.0 ppm (Table 1). Inasmuch as DDE was the only chemical found in high concentrations in the carcass fat before the experiments, it was not surprising that only DDE occurred in high concentrations in the brain. Although younger and older individuals had different chemical body burdens at the start of the experiments, there was no difference in the amounts of residues in the brain at death ($P > .2$, Table 1). Thus, relatively lower amounts

Table 1. Concentrations of organochlorine residues in the brains and carcasses of *Tadarida brasiliensis* from the reference, unexercised, and exercised groups (*24*); ND, not detected. Residues, reported as ND, were entered into the computation as zero.

Value	Residues (ppm, fresh weight basis)							
	Brain				Carcass			
	DDE	DDD	DDT	Dieldrin	DDE	DDD	DDT	Dieldrin
	Reference group, younger animals (N = 8)							
Median	3.7	< 0.02	< 0.02	0.02	92	0.21	0.45	0.19
Range	1.5–17.0	ND–0.03	ND–0.03	ND–0.03	50–300	0.12–0.36	0.27–0.91	0.13–0.41
	Reference group, older animals (N = 5)							
Median	1.3	< 0.02	< 0.02	0.02	22	0.13	0.24	0.12
Range	1.1–11.0	ND–0.04	ND–0.03	ND–0.04	13–66	0.10–0.27	0.19–0.29	0.08–0.23
	Unexercised group, younger animals (N = 2)							
Median	47	0.07	0.20	0.04	75	0.33	0.21	0.06
Range	18–76	ND–0.13	0.11–0.29	ND–0.07	39–110	0.26–0.39	0.17–0.24	ND–0.11
	Unexercised group, older animals (N = 6)							
Median	70	0.12	0.23	0.02	115	0.29	0.25	0.02
Range	10–95	0.06–0.27	0.14–0.55	ND–0.36	16–260	0.18–0.54	0.18–0.53	ND–0.45
	Exercised group, younger animals (N = 5)							
Median	160	0.18	0.41	0.14	130	0.38	0.35	0.14
Range	66–330	ND–0.73	0.21–0.72	ND–0.73	69–270	0.22–1.0	0.14–0.74	ND–0.81
	Exercised group, older animals (N = 5)							
Median	160	0.12	0.38	0.20	150	0.33	0.25	0.21
Range	37–260	ND–0.16	0.28–0.41	ND–0.56	45–460	0.32–0.59	0.17–0.50	ND–0.73

of residues in the carcass (that is, in older animals of the reference group) could present a potential hazard.

Concentrations of DDE and DDT in the brain were significantly higher in the exercised group than in the unexercised group ($P < .05$, Fig. 1 and Table 1). Since the exercised group also lost a significantly greater amount of fat, the amount of pesticide reaching the brain seems to depend on the amount of fat used; however, the experimental design did not indicate whether the stress of exercising per se was influential.

The experimental animals also exhibited a marked increase in the weight of DDT and its metabolites in their brains. However, the weights of DDT in their carcasses decreased although the weights of DDD and DDE increased. This result presumably reflects the chemical breakdown of DDT and the mobilization and redistribution of residues to the brain (*12*).

We witnessed the deaths of two bats in the exercised group; both exhibited the characteristic symptoms of pesticide poisoning (*17*). One, later found to have 260 ppm of DDE in its brain (*18*), was observed on its back with its chest muscles undergoing violent contractions. The second, later found to have 330 ppm of DDE in its brain (*18*), became hyperactive and had intermittent audiogenic seizures that began 3 days before death. These values represent lethal concentrations of DDE in the brain of *T. brasiliensis* and, to our knowledge, constitute the first diagnostic measurements of this toxicant in the brain of a mammal. Residues of DDE in brains of birds killed by this toxicant range from 250 to 660 ppm (wet weight) (*19*). The determination of sublethal and lethal concentrations of DDE is important for interpreting the extent of field contamination because DDE is the chemical usually found in highest concentration in birds and mammals collected in the wild.

Studies with mammals (*11*) and birds (*12*) have shown that clinical illnesses appear when DDT and its metabolites accumulate in the brain from stored body fat during partial or complete food reduction. Our study with young, flying *T. brasiliensis* also demonstrated that significant amounts of organochlorine residues (enough to cause clinical signs of poisoning) reach the brain when carcass fat is depleted under manipulated laboratory conditions. The chemical body burdens in these young were established naturally while the bats were at the maternity roost before their first migratory flight. Although the body burdens in these young are among the highest found in a natural population, higher loads have been reported (*20*).

The chemical body burden and the quantity of body fat used during these flights determine the quantities of pesticides that reach the brain. The amount of fat used by bats during migration is unknown; however, if bats use as much fat as migratory birds (*21*), most of the stored fat is depleted. Banding records indicate that *T. brasiliensis* from Carlsbad Caverns have flown more than 1300 km in 69 days on their southward migration (*22*) and that these bats are capable of flying more than 64 km per night (*1*).

We believe that the stored organochlorine residues in the fat of young *T. brasiliensis* present a serious threat to these bats during their initial migratory flight. Even if the laboratory stress was more severe than the stress of normal migration and lethal concentrations of organochlorine residues are seldom attained during migration, the sublethal potential of these chemicals in the brain, although unknown in bats, could significantly reduce survival (*23*). Further evidence, including the determination of sublethal effects of DDE and the amount of fat used during migration, may confirm the relationship between pesticides and declines in populations of *T. brasiliensis*.

KENNETH N. GELUSO
J. SCOTT ALTENBACH
Department of Biology, University of New Mexico, Albuquerque 87131
DON E. WILSON
National Fish and Wildlife Laboratory, Fish and Wildlife Service, National Museum of Natural History, Washington, D.C. 20560

References and Notes

1. E. L. Cockrum, *Univ. Kans. Mus. Natur. Hist. Misc. Publ. No. 51* (1969), p. 303.
2. R. W. Barbour and W. H. Davis, *Bats of America* (Univ. of Kentucky Press, Lexington, 1969), pp. 1–286.
3. Based on 1.0 g of insects per night per bat for 120 nights → R. B. Davis, C. F. Herreid II, H. L. Short, *Ecol. Monogr.* **32**, 311 (1962)].
4. V. C. Allison, *J. Mammal.* **18**, 80 (1937).
5. J. S. Altenbach, K. N. Geluso, D. E. Wilson, in *Symposium on Biological Investigation of Guadalupe Mountains National Park, Texas* (National Park Service, Washington, D.C., in press).
6. R. F. Reidinger, Jr., thesis, University of Arizona (1972).
7. E. L. Cockrum, *J. Ariz. Acad. Sci.* **5**, 198 (1969); C. E. Mohr, *Bull. Natl. Speleol. Soc.* **34**, 33 (1972).
8. D. R. Clark, Jr., C. O. Martin, D. M. Swineford, *J. Mammal.* **56**, 429 (1975).
9. D. E. Wilson, K. N. Geluso, J. S. Altenbach, *Proc. East Africa Acad.*, in press.
10. L. F. Stickel, in *Environmental Pollution by Pesiticides*, C. A. Edwards, Ed. (Plenum, New York, 1973), p. 254.
11. W. E. Dale, T. B. Gaines, W. J. Hayes, Jr., *Toxicol. Appl. Pharmacol.* **4**, 89 (1962); ———, G. W. Pearce, *Science* 142, 1474 (1963).
→ A. C. Van Velzen, W. B. Stiles, L. F. Stickel, *J. Wildl. Manage.* **36**, 733 (1972).
13. All animals were born during the 1974 reproductive season. Bats having cartilaginous zones clearly visible on both sides of the finger joints were classified as younger individuals; those having one zone visible were classified as older individuals.
14. The brain and carcass (includes the entire body minus the brain, gastrointestinal tract, wings, feet, and skin) of each individual were analyzed for *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, dieldrin, aldrin, heptachlor epoxide, heptachlor, endrin, lindane, chlordane, toxaphene, polychlorinated biphenyls (PCB's), and fat content at the Denver Wildlife Research Center, Colorado. Analytical procedures were modified from a method developed by J. E. Peterson, K. M. Stahl, and D. L. Meeker [*Bull. Environ. Contam. Toxicol.* **15**, 135 (1976)] in that liquid-liquid partitioning was found to be an unnecessary cleanup and was not employed. We identified and quantified the residues by electron-capture gas chromatography, using a chromatograph (Tracor model 560 or 220) with two dissimilar columns (3 percent OV-1 and 5 percent QF-1 on 80/100 mesh Chromasorb W) for confirmation. In the few samples found containing PCB's, DDE could always be quantified; the other chemicals in some cases had peaks with retention times similar to the early eluting peaks of the PCB's and could not be quantified. The limits of sensitivity were 0.5 ppm by weight of fresh (wet) tissue for PCB's, 1.0 ppm for toxaphene, and 0.02 ppm for the other chemicals. Residue data were not adjusted on recoveries given for this method.
15. Frequency curves of pesticide residue values were platykurtic in the experimental groups and positively skewed in the reference group. The Mann-Whitney U test was used to determine the significance of difference in the planned comparisons between the residue data. Student's *t*-test was used to compare data on fat content that had a normal distribution. Two-tailed probabilities were used in both tests. Medians were used to describe the residue data because the distributions were asymmetrical. Because males and females within age groups did not differ in the amounts of fat or residues as determined by statistical tests and direct observations, sexes were pooled within age groups for subsequent comparisons. Age groups were treated separately in comparisons between the reference group and experimental groups. Only one probability level is given for both age classes because the probability levels were identical for the two age classes.
16. Rectal body temperatures were taken with a Schultheis thermometer at 10 a.m. 5 days after capture.
17. M. M. Luckens, in *Pesticides in the Environment: A Continuing Controversy*, W. B. Deichmann, Ed. (Intercontinental Medical Book, New York, 1973), p. 63.
18. The brain of the older bat (260 ppm) also contained 0.12 ppm of DDD, 0.38 ppm of DDT, and 0.56 ppm of dieldrin. The brain of the younger bat (330 ppm) also contained 0.73 ppm of DDD, 0.72 ppm of DDT, and 0.73 ppm of dieldrin.
19. W. H. Stickel, L. F. Stickel, F. B. Coon, in *Pesticides Symposia*, W. B. Deichmann, Ed. (7th Inter-American Conference on Toxicology and Occupational Medicine, Helios and Associates, Miami, 1970), p. 287.
20. B. J. LeBoeuf and M. L. Bonnell, *Nature (London)* **234**, 108 (1971).
21. I. C. T. Nisbet, *Bird-Banding* **34**, 139 (1963).
22. B. Villa R. and E. L. Cockrum, *J. Mammal.* **43**, 43 (1962).
23. For references demonstrating sublethal effects of pesticides in birds, → J. M. Scott, J. A. Wiens, R. R. Claeys, *J. Wildl. Manage.* **39**, 310 (1975).
24. In addition to the organochlorine residues shown in Table 1, Aroclor 1260 (= PCB) was detected in the carcasses and brains of two animals from the unexercised group and two animals from the exercised group. Concentrations ranged from < 0.5 to 1.0 ppm in the brain and < 0.5 to 1.8 ppm in the carcass. One individual from the reference group contained < 0.02 ppm of endrin in its carcass.
25. The cooperation and assistance of the personnel of the National Park Service is appreciated; we especially thank G. M. Ahlstrand, C. Peterson, B. Phillips, P. Van Cleave, and R. H. Wauer. For use of the facilities at the Denver Wildlife Research Center and training in residue analysis, we thank R. E. White and D. L. Meeker. We thank M. A. Bogan, A. L. Gardner, and S. R. Humphrey for critically reviewing this manuscript. Financial support was obtained from the National Park Service, the World Wildlife Fund, and the U.S. Fish and Wildlife Service.

26 April 1976; revised 6 July 1976