sible that it is cytoplasmic material from the protoplasts. The only evidence of this is that nearly all of the agglutinated cells were dead 10 minutes after the addition of elicitor, as shown by staining with fluorescein diacetate (*11*). In contrast, protoplasts incubated with salt solution or any of the commercial polysaccharides at the concentrations shown in Table 1 did not agglutinate the protoplasts, did not cause debris accumulation (Fig. 2), and did not result in cell death.

The remainder of the studies were done with elicitor isolated by Bio-Gel chromatography because substantially lower concentrations of elicitor were required to cause agglutination (21 versus 250 μg of D-glucose equivalents per milliliter). Agglutination occurred when protoplasts (1×10^5 cells per milliliter) were incubated for 10 minutes with this elicitor preparation at a concentration of 21 μg of D-glucose equivalents per milliliter, but not at concentrations lower than 10 μg or higher than 60 μg of D-glucose equivalents per milliliter. The agglutinated cells were dead and had the same appearance as those shown in Fig. 1. The cells incubated with elicitor at high concentrations were killed even though they did not agglutinate.

Laminarin did not agglutinate protoplasts, but it did inhibit elicitor-mediated agglutination when the laminarin was incubated with the protoplasts for 10 minutes prior to the addition of elicitor at a final concentration of 21 μg of D-glucose equivalents per milliliter (Table 2). Laminarin did not reverse agglutination once it had occurred.

The data presented are consistent with the existence of elicitor receptor sites on the outer surface of potato leaf plasma membranes. If this is the explanation of elicitor-mediated agglutination, it is evident that the elicitors isolated by our procedure are multivalent. The proposed receptors are capable of being saturated, as shown by the lack of agglutination with high elicitor concentrations. They are probably specific for β-1,3-glucan portions of the elicitor molecule because laminarin inhibits the agglutination.

The elicitor used in our studies was isolated from a race of *P. infestans* that can easily infect Kennebec potato plants. Thus, our research does not elucidate the mechanism of race specificity. It is possible that race-specific elicitors do exist (*12*), but this character is lost during the isolation of cell walls or elicitor. It is also possible that race specificity is due to other chemicals working in concert with elicitors (*6*), or that elicitors are released from the cell walls of an invading fungus only if the plant being penetrated has a substantial amount of resistance to that race of the fungus.

BRIAN M. PETERS
DAVID H. CRIBBS
DAVID A. STELZIG
Plant Sciences Division, West Virginia University, Morgantown 26506

References and Notes

1. P. Albersheim, A. R. Ayers, Jr., B. S. Valent, J. Ebel, M. Hahn, J. Wolpert, R. Carlson, *J. Supramol. Struc.* **6**, 599 (1977).
2. J. Kuc, *Annu. Rev. Phytopathol.* **10**, 207 (1972).
3. N. Keen, J. Partridge, A. Zaki, *Phytopathology* **62**, 768 (1972).
4. D. Mancarella, thesis, West Virginia University (1976).
5. K. A. Kota and D. A. Stelzig, *Proc. Am. Phytopathol. Soc.* **4**, 216 (1977).
6. A. R. Ayers, B. Valent, J. Ebel, P. Albersheim, *Plant Physiol.* **57**, 766 (1976).
7. A Teflon pestle attached to a motorized drive was used to homogenize (at room temperature) 500 mg of cell wall and 50 ml of 0.05*M* sodium acetate buffer, *p*H 5.0, in a tight-fitting glass tube. When the slurry was homogeneous, 50 mg of laminaranase was added, and the homogenization was continued for 30 seconds.
8. The dialyzate was passed through a column (2 by 55 cm) of Dowex 50W-X8 in the H^+ form, and the column was washed with water until the eluate was nearly neutral. This eluate was then passed through a column (2.5 by 37 cm) of Amberlite IR-45 in the OH^- form, and the column was washed with three bed volumes of water. The combined eluate was then concentrated to 60 ml by flash evaporation at 40°C. In the second method, the dialyzate was first concentrated to 60 ml, and a portion containing 5 mg of carbohydrate was placed on a column (0.9 by 40 cm) of Bio-Gel P-2. The eluate was collected in 2-ml fractions, with water as the eluant. The fractions in the single peak of elicitor held up by the column that contained at least 60 μg of carbohydrate were then pooled. The elicitors isolated by both of these methods caused necrosis and phytoalexin synthesis when added to slices of potato tubers.
9. J. F. Shepard and R. E. Totten, *Plant Physiol.* **60**, 313 (1977).
10. A portion (1 g) of leaflets was surface sterilized, cut into strips (1 by 5 mm), and incubated in the dark for 1 hour with 20 ml of preplasmolyzing solution, composed of 0.1 m*M* $CaCl_2$ in 0.4*M* mannitol. The leaflet strips were then vacuum-infiltrated with 20 ml of enzyme solution containing salts [E. M. Frearson, J. B. Power, E. C. Cocking, *Dev. Biol.* **33**, 130 (1973)], 0.5 percent Cellulysin (Calbiochem), and 0.1 percent pectinase (Sigma) in 0.4*M* mannitol, *p*H 5.6. The flasks were shaken at 84 rev/min at 31°C for 4 hours in the dark. The clusters of tissue were broken up by tapping the flasks and filtered through one layer of Miracloth (Calbiochem). The filtrate was centrifuged at 100*g*, and the protoplasts were purified [D. W. Galbraith and D. H. Northcote, *J. Cell Sci.* **24**, 295 (1977)]. The protoplasts were washed three times with isotonic salt solution (0.2*M* KCl, 0.01*M* $CaCl_2$, and 0.02*M* tris-HCl buffer, *p*H 7.2) and tested for viability (*11*).
11. P. J. Larkin, *Planta (Berl.)* **128**, 213 (1976).
12. N. T. Keen, *Science* **187**, 74 (1975).
13. This is Scientific Paper 1522 of the West Virginia Agricultural Experiment Station, Morgantown.

1 February 1978; revised 11 April 1978

Rabies: Decimation of a Wolf Pack in Arctic Alaska

Abstract. *In a pack of ten wolves, one wolf behaved atypically and fought with several packmates. This wolf was shot when it approached the author. Within 4 weeks at least six other members of the pack were dead. Rabies was confirmed in the wolf that was shot and in two others that had not decomposed. Most of the wolves infected with rabies had sought or remained at familiar areas in the core area of their territory, which implies that they were not contacting neighboring packs. This was confirmed with an aerial survey. Arctic foxes, experiencing a regionwide rabies epizootic, were suspected vectors.*

Rabies, a viral disease affecting the nervous system, causes altered behavior, paralysis, and death in most mammals. It is transmitted by introduction of virus-laden saliva into a bite wound, by ingestion of infected material (*1*), or by inhalation of contaminated air (*2*). Knowledge of the effect of rabies on family groups, behavior of normal animals in contact with rabid conspecifics, and behavior and movements of rabid animals would lead to better understanding of the persistence of rabies in wildlife populations (*3*). In this report I describe the behavior of rabid wolves in the wild.

Reports of rabies in wolves (*Canis lupus*) have dealt almost exclusively with attacks on man (*4*). Rabies has rarely been recorded in wolves in North America. In Alaska, for example, there were only six laboratory-confirmed cases before 1977 (*5–7*). Most rabid wolves in North America were reported during rabies outbreaks (epizootics) in arctic foxes (*Alopex lagopus*) or red foxes (*Vulpes vulpes*) (*5, 8–10*). Wolves seem to be an important reservoir or vector of rabies only in the eastern Mediterranean (*11*).

Rabies is enzootic in fox populations in tundra regions of Alaska (*6, 12, 13*), and in 1976 an epizootic began in arctic foxes along the Arctic Coast (*7*). In the summer of 1977 I documented an outbreak of rabies in a pack of wolves in the upper Hulahula River valley on the north slope of the Brooks Range in northeastern Alaska (Fig. 1). In the summer of 1976 I had studied the effects of human disturbance on this pack (*14*) and in 1977 had begun a study of its behavior. I was in the Hulahula valley from 5 June to 18 July, 2 to 10 August, and 12 August to 12 September 1977. Homesites (*15*) were observed for wolf activity from approximately 1.0 km with a spotting scope for a total of 160 hours between 8 June and 18 July. In June, two wolves were captured, immobilized, fitted with radio-transmitting collars, and released at their cap-

0036-8075/78/0728-0365$00.50/0

ture sites (*16*). Both were frequently located from the ground by means of radio receivers.

Wolf packs are family groups composed of a dominant (alpha) pair, their current young, and any number of offspring of previous years. A pack may be territorial (*17*) or migratory (*18*). In summer, all packs with pups localize near dens and later near rendezvous sites (*15*), areas where pups remain while adults hunt. The Hulahula pack in 1977 consisted of a minimum of eight adults (yearlings and mature adults) and two pups (Table 1). In 1976 it had included eight adults and four pups (*14*). The breeding female (Alpha-F) was the same both years, as was the den and at least the first two of three rendezvous sites (RS-1, RS-2, and RS-3).

Between 2110 and 2225 (Alaska Standard Time) on 13 July at RS-2, I observed fighting that, among wolves in summer, was unusually intense and vicious. A dark tawny wolf (DT), later found to be rabid, aggressively and repeatedly fought Alpha-M, M-95, and SW (Table 1). Wolf F-94, which avoided fighting, was attacked twice by DT, and DT chased and probably bit at least one pup. Wolf DT did not behave submissively when knocked down, as others in similar positions invariably did, but attacked instead. During pauses in the fighting, DT was watched intently by the others as it wandered back and forth over trails and made short runs, activities that were unusual and seemingly aimless.

The wolf DT was probably a member of the Hulahula pack since a wolf matching its description was at RS-2 on 12 July. Its behavior on 13 July was consistent with behavior of rabid dogs (*Canis familiaris*) (*19*). There is one report of a wolf attempting to escape a rabid wolf (*20*), but my observations show that this behavior is not always the case.

Table 1. Characteristics and fates of members of the Hulahula wolf pack.

Wolf	Sex	Weight (kg)	Fate*
DT	Male	29.8	Approached author and was killed on 14 July; rabies-positive
M-95	Male	29.6	Fitted with radio-transmitting collar on 13 June; found dead on 8 August; died about 4 or 5 August; porcupine quills in muzzle; rabies-positive
Alpha-M	Male	41.9	Alpha-male; found dead on 9 August; died about 7 August; rabies-positive
M-02	Male		Found dead on 17 August; died after 2 August
Alpha-F	Female		Mother and alpha-female; found dead on 17 August; died late July or early August; porcupine quills in muzzle
F-94	Female		Fitted with radio-transmitting collar on 15 June; found dead on 13 August; died in late July or early August; one porcupine quill in muzzle
SW			Unaccounted for
Wolf-X			Unaccounted for
Pup-1	Male		Found dead on 13 August; died in late July or early August
Pup-2			Unaccounted for

*Figure 1 shows where the wolves died.

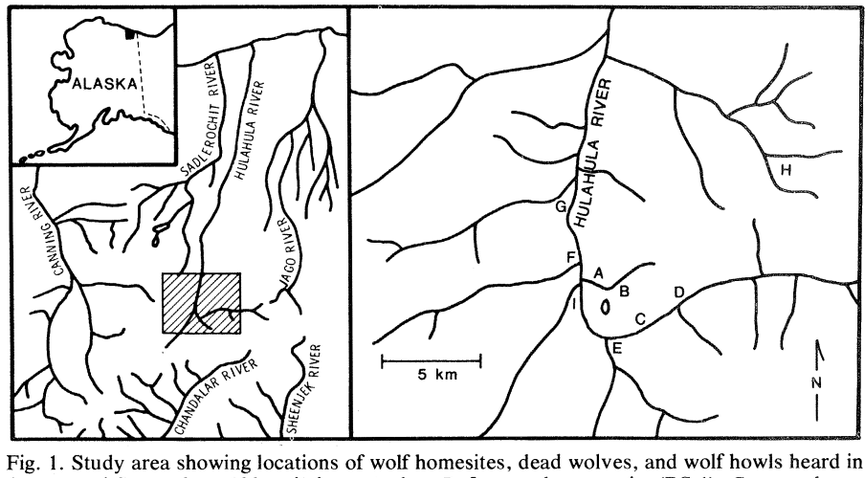

Fig. 1. Study area showing locations of wolf homesites, dead wolves, and wolf howls heard in August and September. Abbreviations: *A*, den; *B*, first rendezvous site (RS-1); *C*, second rendezvous site (RS-2), location of M-02 and Alpha-M; *D*, third rendezvous site (RS-3), location of F-94, Alpha-F, and Pup-1; *E*, where DT was killed after approaching R.C.C.; *F*, location of M-95; *G*, *H*, and *I*, where howls were heard.

At 2120 on 14 July, a wolf passed my tent, and I whistled to attract it for the purpose of taking photographs. After briefly trotting back and forth 10 to 15 m away, it approached to within 3 m. I became apprehensive at this unusual behavior and drove it off by shouting and banging pots together. It returned twice more and each time I struck it in the head with a boot I had picked up. It circled out 10 to 15 m each time it was driven off. I finally killed it with a pistol when it approached again. It had trotted in a slightly staggering manner, not appearing alert, and it had not vocalized. It bit the ground at least once and snapped at the boot the second time I hit it. Considerable saliva and debris covered its muzzle. On the basis of its size and color, I identified the wolf as DT. Six days later, it was found to be rabies-positive by the fluorescent antibody (FA) and mouse inoculation tests (*21*). Its stomach contents, totaling 0.5 liter, included wolf hair, moss, wood chips, and sand.

Between 8 and 17 August, I found six wolves dead in the area (Table 1). Two of them, M-95 and Alpha-M, tested on 11 August, were rabies-positive by the FA test. The other four were too decomposed for testing. None of the carcasses had been scavenged. Apparently all six had died within a 10-day period around 4 August. At least four of them had had contact with DT on 13 July. It was less than 4 weeks from exposure to rabies from DT to the deaths of these wolves, similar to dogs in this respect (*19*).

Three wolves died with porcupine (*Erethizon dorsatum*) quills in their muzzles, as has been reported for rabid red foxes (*10, 13*). Other observations had indicated that normal wolves avoid contacting porcupines.

The rabid wolf DT fought with others at RS-2; two adults and a pup died at RS-3; and two other adults died at RS-2 which was not in use when they died there. These data suggest that rabid wolves tend to seek, or remain at, familiar areas. Wolf M-95 did not die at a homesite, and would not have been found had it not been located with the radio receiver. Other wolves may have died, but I did not find their bodies and I was unable to search even 1 percent of the pack's estimated territory area of 850 km^2 (*14, 22*).

At least three pack members were unaccounted for. One of these (SW) had fought with DT on 13 July, and Pup-2 undoubtedly had had contact with its mother and its littermate who had both died of rabies. Evidence indicates that at least one pack member may have survived beyond 10 August. Dall sheep

(*Ovis dalli*) hunters heard wolf howls on the nights of 9 to 10 and 11 to 12 August, and I heard two howls on 8 September (Fig. 1).

During 20 to 22 March 1978 I conducted 5 hours of aerial survey in a Turbo-Beaver aircraft along the Jago, Okpilak (located between the Jago and Hulahula rivers), Hulahula, and Canning rivers. A complete survey would have required at least 15 hours. Two black wolves and one gray wolf were 11 km north of the den location shown in Fig. 1. A sheep hunter had seen a black wolf in this area in early September 1977. These wolves may represent a pack that existed north of the Hulahula pack prior to the outbreak of rabies; there were no black wolves in the Hulahula pack. Two gray wolves were on the Canning River 48 km west of the den. Tracks of at least two wolves were on the Okpilak River 42 km northeast of the den. Wolf tracks were also seen on the Jago River and in most of the Canning drainage. These findings suggest that only the Hulahula pack was affected by rabies.

Indeed, several factors would make it unlikely in northeastern Alaska for a rabid wolf to infect a wolf in another pack. Wolf densities there are usually lower than one wolf per 180 km^2 (*22*). A rabid wolf, infective for only a few days, would have to leave its home range, then contact and infect another wolf before it died. [There is one report of a rabid wolf traveling at least 24 km after attacking a man (*12*).] Evidence described herein suggests that most rabid wolves seek familiar areas. Supporting data are sparse, but no one has reported significantly lowered wolf densities following local outbreaks of rabies in wolves (*12, 21, 23*), which suggests that pack-to-pack transmission of rabies is uncommon. Predator control, dispersing wolves filling vacant territories, and subsequent reproduction would mask the devastating effects of the disease.

Since there was a rabies outbreak among arctic foxes along the Arctic Coast (*7*), the most probable source of rabies was a rabid arctic fox. Because of a lack of prey on the coastal plain in northeastern Alaska, wolves are rarely seen near the coast. It is not uncommon, however, for arctic foxes to travel south from the coast, particularly when at high densities as they were in 1976 to 1977. A few were even seen south of the Brooks Range during this period (*7*). Moving south brings them in contact with wolves. Wolves will chase and attempt to kill foxes which they encounter (*14, 24*), and in so doing, may be bitten or may eat such a fox. If the fox is rabid, the wolf may become infected. In August at RS-2, I found the skull of an arctic fox showing evidence of chewing by a wolf, and the fox's chipped canines indicated that it may have bitten rocks, not done by normal foxes.

In an earlier incident, three wolves (one confirmed rabid) had attacked men or dogs within a 13-day period in a small area near Aklavik, Northwest Territories, Canada (*8*), which suggests that the three were packmates and were infected about the same time. Because members of wolf packs are socially close, most if not all pack members will be exposed to rabies if one member becomes rabid, decimating the pack. If this is usually true, wolves, which occur in discrete social units (packs) and low densities, would be sporadic hosts of rabies.

RICHARD C. CHAPMAN
Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul 55101

References and Notes

1. J. F. Bell and G. J. Moore, *Am. J. Epidemiol.* **93**, 176 (1971); R. O. Ramsden and D. H. Johnston, *J. Wildl. Dis.* **11**, 318 (1975).
2. W. G. Winkler, in *The Natural History of Rabies*, G. M. Baer, Ed. (Academic Press, New York, 1975), vol. 2, pp. 115–121.
3. E. Preston, *J. Wildl. Manage.* **37**, 501 (1973); B. Toma and L. Andral, *Adv. Virus Res.* **21**, 1 (1977).
4. M. Nikolic, *Z. Tropenmed. Parasitol.* **3**, 283 (1952); L. Gremliza, *ibid.* **4**, 382 (1953); M. Baltazard and M. Ghodssi, *Bull. WHO* **10**, 797 (1954); M. Baltazard and M. Bahmanyar, *ibid.* **13**, 747 (1955); M. Bahmanyar, A. Fayaz, S. Nour-Salehi, M. Mohammadi, H. Koprowski, *J. Am. Med. Assoc.* **236**, 2751 (1976); U. Shah and G. S. Jaswal, *J. Infect. Dis.* **134**, 25 (1976).
5. R. B. Williams, *Can. J. Comp. Med.* **13**, 136 (1949).
6. R. L. Rausch, *Arch. Environ. Health* **25**, 246 (1972).
7. D. Ritter, personal communication.
8. I. M. Cowan, *J. Mammal.* **30**, 396 (1949).
9. G. H. Adlam, *Can. J. Comp. Med.* **17**, 418 (1953); E. E. Ballantyne and J. G. O'Donoghue, *J. Am. Vet. Med. Assoc.* **125**, 316 (1954); P. J. G. Plummer, *Bull. WHO* **10**, 767 (1954); K. F. Wells, *Can. J. Public Health* **48**, 239 (1957).
10. D. H. Johnston and M. Beauregard, *Bull. Wildl. Dis. Assoc.* **5**, 357 (1969).
11. R. K. Sikes, in *Proceedings of the National Rabies Symposium* (National Communicable Disease Center, Atlanta, Ga., 1966), pp. 31–33; in *Infectious Diseases of Wild Mammals*, J. W. Davis, L. H. Karstad, D. O. Trainer, Eds. (Iowa State Univ. Press, Ames, 1970), pp. 3–19.
12. R. Rausch, *J. Wildl. Manage.* **22**, 246 (1958).
13. ———, *Zentralbl. Veterinaermed. B* **22**, 420 (1975).
14. R. C. Chapman, thesis, University of Alaska (1977).
15. P. W. B. Joslin, *Am. Zool.* **7**, 279 (1967).
16. G. B. Kolenosky and D. H. Johnston, *Am. Zool.* **7**, 289 (1967); U. S. Seal and A. W. Erickson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 1410 (1969); L. D. Mech, *Proc. Int. Congr. Game Biol.* **11**, 315 (1974).
17. L. D. Mech, *Am. Zool.* **12**, 642 (1972); *U.S. For. Serv. Res. Rep. NC-97* (1973).
18. E. Kuyt, *Can. Wildl. Serv. Rept. Ser. No. 21* (1972); G. R. Parker, *J. Mammal.* **54**, 341 (1973).
19. C. C. Rife, in *Canine Medicine*, E. J. Catcott, Ed. (American Veterinary Publications, Wheaton, Ill., 1968), pp. 133–144; E. S. Tierkel, in *The Natural History of Rabies*, G. M. Baer, Ed. (Academic Press, New York, 1975), vol. 2, pp. 123–137.
20. R. Rausch, *Arctic* **4**, 147 (1951).
21. P. Atanasiu, in *The Natural History of Rabies*, G. M. Baer, Ed. (Academic Press, New York, 1975), vol. 1, pp. 373–400; R. E. Kissling, in *ibid.*, pp. 401–416. Diagnostic tests were performed by the Virology-Rabies Unit, Alaska Division of Public Health, University of Alaska Campus, Fairbanks.
22. R. O. Stephenson and J. J. Sexton, *Wolf Report* (Alaska Department of Fish and Game, Juneau, 1974), mimeographed report.
23. R. J. Rutter and D. H. Pimlott, *The World of the Wolf* (Lippincott, Philadelphia, 1968).
24. L. D. Mech, *The Wolf* (Doubleday, New York, 1970).
25. I thank the following individuals for their help during summer 1977: L. D. Mech, P. Gipson, C. Curby, D. Ritter, D. DeVries, pilots W. Audi of Kaktovik and D. Butler of Fort Yukon, and the sheep hunters and guides who used the Hulahula River. I thank the U.S. Fish and Wildlife Service and the Alaska Department of Fish and Game for permits to conduct the radio-telemetry study. I thank the Arctic National Wildlife Range for financial support in August through October 1977 and for logistic support in March 1978. I thank the Dayton Natural History Fund of the Bell Museum of Natural History for financial support in March, and L. D. Mech and P. Jordan for criticizing this manuscript.

9 February 1978; revised 17 April 1978

Retrograde Amnesia Produced by Several Treatments: Evidence for a Common Neurobiological Mechanism

Abstract. *This experiment examined the effects on memory of various amnestic treatments in animals earlier treated with the α-adrenergic antagonist phenoxybenzamine (PBZ). Thirty minutes before being trained in a one-trial inhibitory (passive) avoidance task, animals received an injection of PBZ or saline. Immediately after training, each animal received one of the following amnestic treatments: stimulation of the frontal cortex or amygdala, pentylenetetrazol, diethyldithiocarbamate, or cycloheximide. In control animals, each treatment produced retrograde amnesia. However, PBZ-treated animals did not develop amnesia. These findings suggest that there may be a common neurobiological mechanism underlying the amnesias produced by many treatments.*

During the past 30 years, various treatments have been used to produce retrograde amnesia in animals (*1*). In many studies, a specific amnestic treatment was chosen because of a particular known neurobiological response to it. These studies represent indirect attempts to learn about the nature of memory storage processing by relating posttraining interference with the activity of certain neurobiological systems to deficits in later retention (*2*). Thus, the amnesias produced by electroconvulsive shock, some forms of direct electrical

0036-8075/78/0728-0367$00.50/0