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Consideration of Inactivated Rabies Vaccines as Oral Immunogens of Wild Carnivores

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ABSTRACT: An experimental β-propiolactone (BPL)-inactivated rabies virus vaccine was evaluated for the oral immunization of captive raccoons (*Procyon lotor*) and red foxes (*Vulpes vulpes*). None of 10 red foxes administered a single 1.0 ml dose of BPL-inactivated rabies virus vaccine (PM strain; 100 or 500 μg protein) *per os* developed detectable anti-rabies virus-neutralizing antibodies (VNA) at any time over 8 wk of observation. Foxes were excluded from further study. In two different groups of five to six raccoons, each administered a single 1.0 ml dose of BPL-inactivated rabies virus vaccine (ERA strain) *per os*, at concentrations of 100 or 400 μg protein, only a single animal in each group demonstrated evidence of seroconversion within 4 wk. In contrast, instillation of a single dose (500 μg protein) of BPL-inactivated rabies virus vaccine (ERA strain), directly into the small intestine via fiberoptic endoscope, or ERA vaccine (800 μg protein) instillation to the buccal cavity by needle-less syringe, resulted in the production of rabies-specific VNA and protection against lethal rabies infection in three of six, and in four of six raccoons, respectively; all seven control raccoons succumbed to street virus challenge. These preliminary challenge studies, while somewhat encouraging, demonstrate that considerable quantities of purified viral antigen are required for even minimal oral efficacy against lethal rabies infection. At the present time, therefore, potent, self-replicating, attenuated, or recombinant viruses offer the most versatile, economic, efficacious, and safe solutions to terrestrial rabies control of free-ranging carnivores.

Key words: Rabies, oral vaccination, raccoon, red fox, recombinant vaccine, *Vulpes vulpes*, *Procyon lotor*.

The mid-Atlantic raccoon (*Procyon lotor*) rabies epizootic, which began as a single focus in late 1977 (Centers for Disease Control, 1982; Jenkins et al., 1988), is one of the most intensive wildlife rabies outbreaks recorded in the United States. It currently affects the states of West Virginia, Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, and the District of Columbia. Once the outbreak extends from southern Virginia into North Carolina, raccoon rabies will encompass a geographic belt from New England to the southeastern region of the United States, previously affected by enzootic raccoon rabies. As raccoon rabies entrenchment proceeds along this heavily populated corridor, human rabies exposures are expected to increase (Centers for Disease Control, 1988).

Conventional rabies control approaches, such as mandatory enforcement of companion animal rabies vaccination and intensified public education concerning the dangers of exposure to wildlife, are indirect methods only, aimed at protection of human and domestic animal populations. In theory, the only direct, ethical, cost-effective, long-term management of large, contiguous, enzootic terrestrial wildlife rabies areas is the oral vaccination of free-ranging carnivores (Baer, 1988) through the strategic distribution of vaccine-laden baits (Johnston et al., 1988). Although the efficacy of both orthopox virus recombinant (Rupprecht et al., 1986; Esposito et al., 1988) and attenuated (Rupprecht et al., 1989) rabies vaccines for raccoons has been demonstrated in the laboratory, initial biological safety concerns, either potentially realistic (Rupprecht et al., 1990) or speculative (Rupprecht, 1990), have significantly delayed widespread deployment of such vaccines intended for field control of the mid-Atlantic raccoon rabies outbreak. Concomitant with laboratory safety and efficacy evaluations of a vaccinia-rabies

glycoprotein (V-RG) recombinant virus vaccine (Rupprecht and Kieny, 1988) for raccoons, and ongoing plans for additional V-RG recombinant virus vaccine field trials in North America (Hanlon et al., 1989; Hable et al., 1992), an alternative experimental approach was taken to critically evaluate the utility of inactivated rabies virus vaccines, administered by the oral or enteric routes to captive wild carnivores, as potential immunogens.

To accomplish this objective, the fixed rabies viruses PM and ERA (The Wistar Institute Virus Collection, Philadelphia, Pennsylvania, USA) were each propagated on BHK-21 cell monolayers, grown at 37 C in Eagle's minimum essential medium supplemented with 10% fetal calf serum (Wiktor, 1973). Cell culture supernatants were harvested and inactivated by β-propiolactone (BPL) treatment (1:4,000) (Wiktor et al., 1972). The virus suspensions were then concentrated and purified by zonal ultra-centrifugation on a sucrose gradient (Wiktor et al., 1977). Each purified virus was suspended in phosphate-buffered saline and was adjusted to a protein concentration of 1.0 mg/ml. Routine incubation of each vaccine upon BHK-21 cell monolayers, followed by blind passages of these cell culture fluids onto BHK-21 cells, and the intracerebral inoculation of 4- to 6-wk-old ICR mice (Harlan Sprague Dawley, Indianapolis, Indiana, USA), confirmed the absence of active virus in the vaccine preparations.

Animals consisted of rabies sero-negative adult foxes (*Vulpes vulpes*), red genotype, and raccoons, 1 to 5 yr of age, kindly supplied by the Ontario Ministry of Natural Resources (Maple, Ontario, Canada). Prior to handling, all animals were sedated by the intramuscular administration of 0.5 to 1.0 ml of ketamine hydrochloride (Rogar/STB, Inc., London, Ontario, Canada) at 100 mg/ml. Both foxes and raccoons were given rabies viral protein *per os* by needle-less syringe; however, only foxes received the PM virus, and only raccoons received ERA vaccine orally and additionally by the enteric route, as follows. Foxes were sedated and five each were administered either 100 μg or 500 μg of the PM rabies virus vaccine, *per os*. In a similar manner, three groups of raccoons, five to six animals each, were sedated and administered a single 1.0 ml dose of either 100 μg, 400 μg, or 800 μg of the ERA rabies vaccine, *per os*. A fourth raccoon group received 0.5 ml of the ERA rabies vaccine (500 μg) by instillation directly into the duodenum. Vaccine was applied through a catheter threaded into the small intestine, from a fiberoptic endoscope which had been introduced through the animal's mouth into the stomach, following appropriate antiemetic and antisalivation treatment, as previously described in detail for foxes (Lawson et al., 1989). Blood samples were collected from foxes and raccoons at 0, 14, 30, and 60 or 90 days after vaccine administration. Sera were stored at −20 C prior to analysis for rabies virus-neutralizing antibodies (VNA) by the modified rapid fluorescent focus-forming inhibition microtest (Zalan et al., 1979), using ERA virus as challenge, with results calculated as the reciprocal of the highest two-fold dilution resulting in virus neutralization. Seroconversion was considered to occur on the basis of at least a four-fold rise in comparison of paired test sera. Animals were observed daily for clinical signs of illness resulting from vaccination.

All animals remained healthy throughout the observation period. In contrast to raccoons receiving larger doses of ERA vaccine, none of the 10 red foxes administered the PM vaccine *per os* developed rabies VNA by day 60; foxes, therefore, were excluded from further study. Similarly, in each of the raccoon groups given lower doses of ERA vaccine *per os* at 100 μg or 400 μg, only a single animal each developed rabies VNA titers of ≥32. Considering this rather poor antibody response, these two raccoon groups also were excluded from additional study. Such negative findings corroborate previous research (Debbie et al., 1972; Black and

Lawson, 1973; Lawson et al., 1989) that documented the lack of immunogenicity of other inactivated rabies viral antigens delivered via the buccal route to red foxes. While Baer (1975) reported that inactivated nervous tissue vaccine protected six of 14 foxes against rabies, the use of an explosive "coyote-getter" device to deliver the vaccine could be considered akin to parenteral vaccination because of the resulting damage to buccal mucosa.

In the six raccoons administered 500 μg of BPL-inactivated ERA rabies vaccine via endoscope, three animals demonstrated seroconversion by day 14 (Group 1, Table 1), with VNA gradually declining within 3 mo. Only these three raccoons with demonstrable VNA from the enteric vaccination group survived lethal intramuscular challenge on day 90 with $1 \times 10^{5.5}$ $MICLD_{50}$/ml of street rabies virus strain MD5951 (Rupprecht et al., 1989), whereas all control raccoons succumbed. In comparison, four of six raccoons administered a single 800 μg dose of BPL-inactivated ERA rabies virus vaccine *per os* demonstrated rabies-specific VNA by day 14. These VNA persisted for at least 3 mo. The two other vaccinated raccoons, without evidence of any seroconversion, succumbed to experimental challenge (Group 2, Table 1); surviving vaccinates remained healthy until the time of routine euthanasia by intravenous barbiturate overdose 90 days post-challenge. Serological response post-challenge was not specifically measured. All surviving vaccinates, as opposed to all seven controls and the five vaccinated raccoons which succumbed to experimental challenge, were negative for rabies virus antigen in brain material examined at necropsy, using the fluorescent antibody test as described by Goldwasser and Kissling (1958).

It is not known if the foxes and raccoons given lower concentrations of vaccine *per os* would have been protected against rabies, in lieu of primary VNA development. Whereas the higher concentrations of ERA vaccine were incompletely effective in producing 100% seroconversion or protection, it is unlikely that these other, less concentrated preparations contained sufficient antigenic mass for prevention of lethal disease. While other self-replicating rabies virus vaccines administered *per os* to raccoons may adequately prime some animals against street virus challenge without detectable levels of VNA (Rupprecht et al., 1989), insufficient information is available as to the specific comparative immunogenic mechanisms of modified live versus inactivated oral rabies vaccines. Although inherent biological differences in immunological effector response also may be related to host species or rabies vaccine strain (Dietzschold et al., 1987), this facet could not be directly explored because foxes were not given inactivated ERA vaccine by either route, or inactivated PM vaccine by enteric means, and PM vaccine was not tested in raccoons. Additionally, the response to inactivated vaccine administered

TABLE 1. Anti-rabies virus-neutralizing antibody (VNA) titers and response to rabies challenge[a] in raccoons (*Procyon lotor*) given a single dose of inactivated purified rabies virus vaccine via endoscope or *per os*.

	Rabies VNA titers				
	Day 0	Day 14	Day 30	Day 90	Response to challenge
Group 1. Endoscope (500 μg)					
Animal number					
3	≤8	256	512	64	Survived
13	≤8	≤8	≤8	≤8	Died (day 24)
18	≤8	32	32	≤8	Survived
25	≤8	32	32	≤8	Survived
26	≤8	≤8	≤8	≤8	Died (day 19)
27	≤8	≤8	≤8	≤8	Died (day 25)
Group 2. Oral (800 μg)					
4	≤8	32	64	128	Survived
5	≤8	256	512	256	Survived
6	≤8	64	128	128	Survived
12	≤8	≤8	≤8	≤8	Died (day 19)
19	≤8	≤8	≤8	≤8	Died (day 25)
28	≤8	32	64	32	Survived

[a] Raccoons were inoculated intramasseter on day 90 with 0.3 ml ($1 \times 10^{5.5}$ $MICLD_{50}$/ml) of street rabies virus strain MD5951 challenge, in which all control raccoons ($n = 7$) succumbed in 18 to 25 days.

via baits also was not evaluated; immunization *per os* may be quite different than immunization by baits.

Other workers (Nicholson and Bauer, 1981; Atanasiu et al., 1982; Lawson et al., 1982; Brochier et al., 1985; Campbell et al., 1985; Crick et al., 1985; Lawson et al., 1989) have suggested that oral or enteric vaccination with rabies antigen in a variety of species may result in various levels of seroconversion. However, the data contained herein, albeit limited, are the first experimental demonstration of the potential protective efficacy against lethal rabies challenge of orally administered inactivated rabies virus vaccines in a relevant carnivore host. For example, Lawson et al. (1982, 1989) showed that rabies VNA was induced in some red foxes following the administration of two doses of rabies antigen directly to the lumen of the duodenum and that enteric administration of antigen produced an anamnestic response in previously vaccinated foxes. Unfortunately, none of those foxes was challenged with rabies virus. Similarly, Brochier et al. (1985) reported that even red fox cubs that did develop rabies VNA from oral inactivated vaccine administration still succumbed to experimental challenge. Thus, the distinction of rabies vaccine efficacy should be based on utility of protection against disease in the natural host under study, not merely the capability to elicit VNA.

Despite the protective capacity of inactivated vaccine, the large concentrations of purified viral antigen apparently required to induce systemic immunity by the oral route may render this method impractical for use in the large-scale control of rabies in free-ranging carnivores at the present time. For example, most modified live rabies viruses used for oral immunization range in concentrations of $1 \times 10^{7.0}$ to $10^{8.0}$ pfu/ml (Baer, 1988; Debbie et al., 1972; Lawson et al., 1982; Prevec et al., 1990; Rupprecht and Kieny, 1988; Rupprecht et al., 1989; Wandeler et al., 1988). In contrast, it is estimated that viral concentrations of 1×10^{10}pfu/ml or higher would be required to routinely manufacture sufficient harvest for an adequate inactivated oral immunogen by today's production methods. Whereas use of lower titered modified live vaccines for European red foxes are cost-effective when considered against the actual costs incurred otherwise for rabies control (Schneider et al., 1988), the amount of equivalent inactivated vaccine *per os* would probably be prohibitively expensive.

Little is known concerning the mechanisms of vaccination with purified proteins administered via the buccal route. Gastrointestinal contents are highly deleterious to viral antigens once the stomach is reached (Baer, 1988; Lawson et al., 1989); thus, the oro-pharyngeal cavity appears to be the primary site of oral immunization. Considering the additional system components that would be required for successful post-gastric immunization, enteric vaccination, by conservation and transport of highly concentrated antigen through the gastro-intestinal tract to a distal site of immuno-competent cells (e.g., Peyer's patches), is clearly not the method of choice until such time that novel economic methodologies have been thoroughly designed and tested (Wandeler et al., 1988).

Currently, only attenuated (Rupprecht et al., 1989) or othopox virus recombinant (Rupprecht et al., 1986, 1988) rabies vaccines are at a sufficient developmental stage to be seriously considered for field testing of safety and efficacy in naturally-affected mammalian rabies hosts, such as the raccoon. Alternative vaccine vectors, such as recombinant adenovirus vaccines (Prevec et al., 1990; Charlton et al., 1992) may offer additional promise. Broad-based concerns over environmental safety of rabies biologicals in general, such as the potential for vaccine-induced rabies (Rupprecht et al., 1990) or untoward effects resulting from the release of genetically modified viruses, should be measured against social, political, and economic benefits. While the concept of risk minimization is certainly desirable, it is difficult to envision totally

"risk-free" vaccines, even with inactivated vaccines, which could produce toxic or allergic side effects. Moreover, the limited efficacy observed in the laboratory to date would be impractical for use in a field control program, where maximum herd immunity of long duration is necessary (Wandeler et al., 1988).

To be tenable, future research directed towards application of inactivated vaccines for oral vaccination should concentrate heavily upon several critical areas, including: the strategic use of adjuvants (Maharaj et al., 1986) or gastro-intestinal protectants (Brochier et al., 1985; Lawson et al., 1989) for antigen conservation; the construction of additional subunit vaccines, with the potential for diminishing the large concentration of specific immunogen required *per os*, while retaining immunogenicity, such as iscoms (Morein et al., 1984) or immunosomes (Sureau and Perrin, 1988); the feasibility of other recombinant expression vector systems, such as baculoviruses (Prehaud et al., 1989; Fu et al., 1991), for maximum utility in the economic production of the large quantities of viral antigen needed for the protection of multiple mammalian target species against antigenically diverse lyssavirus infections; and those particular mechanisms involved with antigen reception, ingestion, processing, presentation, recognition, and stimulation of vaccinal cell-mediated immunity (Celis et al., 1990) by the oral and enteric routes. Lastly, inactivated vaccine preparations which prove efficacious when delivered *per os* in the laboratory must also be incorporated into compatible edible baits, and similarly tested, in order to be of any practical significance.

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LITERATURE CITED

ATANASIU, P., T. METIANU, AND A. BOLANOS. 1982. Evaluation d'une vaccination rabique experimentale par la voie orale et intestinale avec des vaccins tués concentrés et non-concentrés. Comparative Immunology, Microbiology and Infectious Diseases 5: 187–191.

BAER, G. M. 1975. Wildlife vaccination. *In* The natural history of rabies, G. M. Baer (ed.). Academic Press, New York, New York, pp. 261–266.

———. 1988. Oral rabies vaccination: An overview. Reviews of Infectious Diseases 10: S644–648.

BLACK, J. G., AND K. F. LAWSON. 1973. Further studies of sylvatic rabies in the fox (*Vulpes vulpes*). Vaccination by the oral route. Canadian Veterinary Journal 14: 206–211.

BROCHIER, B., J. GODFROIS, F. COSTY, J. BLANCOU, AND P. P. PASTORET. 1985. Vaccination of young foxes (*Vulpes vulpes, L.*) against rabies: Trials with inactivated vaccine administered by oral and parenteral routes. Annales de la Recherche Medicale 16: 327–333.

CAMPBELL, J. B., I. MAHARAJ, AND J. ROITH. 1985. Vaccine formulations for oral immunization of laboratory animals and wildlife against rabies. *In* Rabies in the tropics, E. Kuwert, C. Mérieux, H. Koprowski, and K. Bögel (eds.). Springer-Verlag, New York, New York, pp. 285–293.

CELIS, E., C. E. RUPPRECHT, AND S. A., PLOTKIN. 1990. New and improved vaccines against rabies. *In* New generation vaccines, G. C. Woodrow and M. M. Levine (ed.). Marcel Dekker, Inc., New York, New York, pp. 419–438.

CENTERS FOR DISEASE CONTROL. 1982. Rabies: Mid-Atlantic States. Morbidity and Mortality Weekly Report 31: 592–593.

———. 1988. Rabies surveillance annual summary: 1987. Atlanta, Georgia, p. 27.

CHARLTON, K. M., M. ARTOIS, L. PREVEC, F. L. GRAHAM, J. B. CAMPBELL, G. A. CASEY, A. I. WANDELER, AND J. ARMSTRONG. 1992. Oral rabies vaccination of skunks and foxes with a recombinant human adenovirus. Archives of Virology. In press.

CRICK, J., F. BROWN, A. A., KING, E. W. WILLIAMS, G. THOMPSON, AND A. J. FEARNE. 1985. Control of wildlife rabies. *In* Rabies in the tropics, E. Kuwert, C. Mérieux, H. Koprowski, and K. Bögel (eds.). Springer-Verlag, New York, New York, pp. 294–301.

DEBBIE, J. G., M. K. ABELSETH, AND G. M. BAER. 1972. The use of commercially available vac-

cines for the oral vaccination of foxes against rabies. American Journal of Epidemiology 96: 231–235.

DIETZSCHOLD, B., M. TOLLIS, C. E. RUPPRECHT, E. CELIS, AND H. KOPROWSKI. 1987. Antigenic variation in rabies and rabies-related viruses: Cross-protection independent of glycoprotein-mediated virus-neutralizing antibody. The Journal of Infectious Diseases 156: 815–822.

ESPOSITO, J. J., J. C. KNIGHT, J. H. SHADDOCK, F. J. NOVEMBRE, AND G. M. BAER. 1988. Successful oral rabies vaccination of raccoons with raccoon pox virus recombinants expressing rabies virus glycoprotein. Virology 165: 313–316.

FU, Z. F., B. DIETZSCHOLD, C. L. SCHUMACHER, W. H. WUNNER, H. C. J. ERTL, AND H. KOPROWSKI. 1991. Rabies virus nucleoprotein expressed in and purified from insect cells is efficacious as a vaccine. Proceedings of the National Academy of Science of the United States of America 88: 2001–2005.

GOLDWASSER, R. A., AND R. E. KISSLING. 1958. Fluorescent antibody staining of street and fixed rabies virus antigen. Proceedings of the Society for Experimental Biology and Medicine 98: 219–223.

HABLE, C. P., A. N. HAMIR, D. E. SNYDER, R. JOYNER, J. FRENCH, V. NETTLES, C. HANLON, AND C. E. RUPPRECHT. 1992. Prerequisites for oral immunization of free-ranging raccoons (*Procyon lotor*) with a recombinant rabies virus vaccine: Study site ecology and bait system development. Journal of Wildlife Diseases 28: 64–79.

HANLON, C. L., D. E. HAYES, A. N. HAMIR, D. E. SNYDER, S. JENKINS, C. P. HABLE, AND C. E. RUPPRECHT. 1989. Proposed field evaluation of a rabies recombinant vaccine for raccoons (*Procyon lotor*): Site selection, target species characteristics, and placebo baiting trials. Journal of Wildlife Diseases 25: 555–567.

JENKINS, S. A., B. D. PERRY, AND W. G. WINKLER. 1988. Ecology and epidemiology of raccoon rabies. Reviews of Infectious Diseases 10: S620–625.

JOHNSTON, D. H., D. R. VOIGT, C. D. MACINNES, P. BACHMANN, K. F. LAWSON, AND C. E. RUPPRECHT. 1988. An aerial baiting system for the distribution of attenuated or recombinant rabies vaccines for foxes, raccoons, and skunks. Reviews of Infectious Diseases 10: S660–664.

LAWSON, K. F., D. H. JOHNSTON, J. M. PATTERSON, J. G. BLACK, A. J. RHODES, AND E. ZALAN. 1982. Immunization of foxes *Vulpes vulpes* by the oral and intramuscular routes with inactivated rabies vaccines. Canadian Journal of Comparative Medicine 46: 382–385.

———, ———, ———, R. HERTLER, J. B. CAMPBELL, AND A. J. RHODES. 1989. Immunization of foxes by the intestinal route using an inactivated rabies vaccine. Canadian Journal of Veterinary Research 53: 56–61.

MAHARAJ, I., K. J. FROH, AND J. B. CAMPBELL. 1986. Immune responses of mice to inactivated rabies vaccine administered orally: Potentiation by *Quillaja* saponin. Canadian Journal of Microbiology 32: 414–420.

MOREIN, B., B. SUNDQUIST, S. HOGLUND, K. DALSGAARD, AND A. OSTERHAUS. 1984. Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. Nature (London) 308: 457–460.

NICHOLSON, K. G., AND S. P. BAUER. 1981. Enteric inoculation with ERA rabies virus: Evaluation of a candidate wildlife vaccine in laboratory rodents. Archives of Virology 67: 51–56.

PREHAUD, C., K. TAKEHARA, A. FLAMAND, AND D. H. L. BISHOP. 1989. Immunogenic and protective properties of rabies virus glycoprotein expressed by baculovirus vectors. Virology 173: 390–399.

PREVEC, L., J. B. CAMPBELL, B. S. CHRISTIE, L. BELBECK, AND F. L. GRAHAM. 1990. A recombinant human adenovirus vaccine against rabies. The Journal of Infectious Diseases 161: 27–30.

RUPPRECHT, C. E. 1990. Vaccinia vectored vaccines. *In* 62nd Annual Western States Veterinary Conference, Proceedings of the Section on Biotechnology and Applications in Veterinary Practice. United States Department of Agriculture, Animal and Plant Health Inspection Service, Hyattsville, Maryland, pp. 25–30.

———, AND M. P. KIENY. 1988. Development of a vaccinia-rabies glycoprotein recombinant virus vaccine. *In* Rabies: Developments in veterinary virology, J. B. Campbell and K. M. Charlton (eds.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 335–364.

———, T. J. WIKTOR, D. H. JOHNSTON, A. N. HAMIR, B. DIETZSCHOLD, W. H. WUNNER, L. T. GLICKMAN, AND H. KOPROWSKI. 1986. Oral immunization and protection of raccoons (*Procyon lotor*) with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proceedings of the National Academy of Science of the United States of America 83: 7949–7950.

———, A. N. HAMIR, D. H. JOHNSTON, AND H. KOPROWSKI. 1988. Efficacy of a vaccina-rabies glycoprotein recombinant virus vaccine in raccoons (*Procyon lotor*). Reviews of Infectious Diseases 10: S803–809.

———, B. DIETZSCHOLD, J. H., COX, AND L. G. SCHNEIDER. 1989. Oral vaccination of raccoons (*Procyon lotor*) with an attenuated (SAD-B19) rabies virus vaccine. Journal of Wildlife Diseases 25: 548–554.

———, K. M., CHARLTON, M. ARTOIS, G. A. CASEY, W. A. WEBSTER, J. B. CAMPBELL, K. F. LAWSON,

AND L. G. SCHNEIDER. 1990. Ineffectiveness and comparative pathogenicity of attenuated rabies virus vaccines for the striped skunk (*Mephitis mephitis*). Journal of Wildlife Diseases 26: 99–102.

SCHNEIDER, L. G., J. H. COX, W. W. MÜELLER, AND K.-P. HOHNSBEEN. 1988. Current oral rabies vaccination in Europe: An interim balance. Reviews of Infectious Diseases 10: S654–659.

SUREAU, P., AND P. PERRIN. 1989. The use of immunosome technology for vaccines against rabies and other viral diseases. European Journal of Epidemiology 5: 272–278.

WANDELER, A. I., S. CAPT, A. KAPPELER, AND R. HAUSER. 1988. Oral immunization of wildlife against rabies: Concept and first field experiment. Reviews of Infectious Diseases 10: S649–653.

WIKTOR, T. J. 1973. Tissue culture methods. *In* Laboratory techniques in rabies, M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, pp. 101–123.

———, H. G. AALESTAD, AND M. M. KAPLAN. 1972. Immunogenicity of rabies virus inactivated by β-propiolactone, acetylethyleneimine and ionizing irradiation. Applied Microbiology 23: 914–918.

———, B. DIETZSCHOLD, R. N. LEAMNSON, AND H. KOPROWSKI. 1977. Induction and biological properties of defective interfering particles of rabies virus. Journal of Virology 21: 626–635.

ZALAN, E., C. WILSON, AND D. PUKITIS. 1979. A microtest for the quantitation of rabies virus-neutralizing antibodies. Journal of Biological Standardization 7: 213–220.

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