Recent Advances in Oral Rabies Vaccination

John G. Debbie
Division of Laboratories and Research, New York
State Department of Health, Albany, NY 12201

The ability to successfully and consistently vaccinate foxes, dogs, and raccoons with modified live virus rabies vaccine (ERA) in the liquid form is an accomplished fact. However, methods of incorporating the vaccine virus into an acceptable bait for field distribution remains a major stumbling block to progress in the practical application of oral rabies vaccination. In addition, we have been unsuccessful in vaccinating skunks against rabies by administering either liquid or lyophilized vaccine directly into the mouth.

Before going into detail on our most recent studies with enteric coated vaccine, I would like to pull together some of the investigations that have brought us this far. Development of an oral vaccine for field application requires solving four major obstacles; namely vaccine efficacy, stability, bait incorporation and safety. Early investigations determined that the ERA strain of rabies vaccine produced the most efficient vaccine but then only at a mouse intracerebral lethal dose 50% ($MICLD_{50}$) titer of approximately $10^{4.5}$/0.03 ml or greater. Titers of the magnitude of 10^{5}-10^{7} $MICLD_{50}$/0.03 ml could only be consistently achieved by propagating the ERA strain on various tissue culture cell lines or in suckling mice. This was accomplished with apparent safety by the oral route to the target species but not without some lethality to certain small rodent species.

Stability was achieved most successfully by incorporating a minimum of 4% casein hydrolysate with the vaccine. Successful stability is considered to be when the vaccine fails to lose over 1 log of virus titer when held at a constant temperature of 22°C for a minimum of 14 days.

Lyophilized ERA rabies vaccine given directly into the mouth of foxes and skunks has produced inconsistent and generally poor results. Liquid vaccine has been successful with foxes and dogs but problems are encountered when one attempts to incorporate the liquid in a bait. Winkler et al. (1975) were able to vaccinate 15 of 36 red foxes by feeding commercial dog biscuits impregnated with liquid vaccine and Winkler and Baer (1976) successfully vaccinated foxes by incorporating liquid vaccine into a plastic straw embedded in a sausage bait. Debbie (1974) vaccinated foxes by feeding chicken eggs injected via the yolk sac with 1 ml of ERA vaccine grown in PK-15 cells (ERA-PK15). A greater percentage of serological conversions in foxes has been demonstrated by feeding them eggs impregnated with ERA virus vaccine passaged in suckling mouse brain. Recent success with vaccine-inoculated eggs fed to raccoons suggests this method may have some field practicality since raccoons do apparently relish eggs in the wild (Nelson and Linder, 1972).

The lack of success with lyophilized rabies vaccine by the oral route and the inability to vaccinate skunks in any form by the oro-pharyngeal route, led to investigations of the enteric route as a possible method of vaccination. Dogs were subjected to surgical manipulation of a gastric tube into the duodenum and commercial ERA vaccine was injected directly into the intestine. One dog was euthanized each day post-inoculation for 5 days and portions of Peyers patches of the duodenum were examined by the fluorescent antibody test (FAT). Small amounts of specific antigen were observed by the FAT in duodenal tissue in the two dogs sacrificed on day 4 and 5. Two additional dogs, which

were not euthanized, developed demonstrable antibody titers within 1-month post inoculation. On the basis of this data, 100 mgm of lyophilized ERA-PK15 vaccine was placed in gelatin capsules, which were subsequently coated with cellulose acetate phthalate.[1] Two coated capsules were given to each of six foxes and three skunks. Two foxes and one skunk developed demonstrable serum neutralizing (SN) antibody titers within one month although the SN titers never exceeded 1/5.

It has been reported by Ferris (1974) in studies with porcine transmissible gastroenteritis vaccine that the use of an enteric-coated capsule of greater than 2.0 mm diameter is not a reliable method of delivering a heat sensitive biological product to the duodenum of an animal. Under this premise, we have been investigating the coating of vaccine virus onto sugar nonpareils (SNP) of 1.5 mm diameter followed by an enteric-coating with hydroxyprophyl methylcellulose phthalate[2] (HMP).

The coating process employs the Wurster air suspension apparatus[3], which operates on the principle of cycling particles in an air stream and spraying these particles while they are suspended.

The vaccine used in our experiments has been the ERA strain passed twice in suckling mice (ERA-SMB) and having a titer of $\geq 10^{6.0}$MICLD$_{50}$/0.03 ml. Initially, a 10% suspension of lyophilized ERA-SMB containing 4% casein was prepared in 5% Penford gum 280[4] (a hydroxy ethyl ether derivative of corn starch).

This solution was sprayed onto 750 grams of SNP at a temperature between 25 and 32°C over a 5-hour time interval. The second coat consisted of a 5% solution of HMP in equal volumes of methylene chloride and absolute ethyl alcohol at the same temperature over a 1-hour interval. Dosage of vaccine was calculated on the basis of 1 ml of a 10% ERA-SMB suspension being one "fox dose". The amount of ERA-SMB solution applied to the 750 grams of SNP calculated to 0.7 gm of coated SNP as being equivalent to one "fox dose".

Virus titrations in 10-12-gram mice were performed on 1 ml of the vaccine-starch solution prior to coating (titration I), on 0.4 gm of virus coated SNP dissolved in 1 ml of sealine (titration II), and on 0.4 gm of the enteric coated SNP dissolved in 1 ml of diluent (titration III). Titration I had a value of $10^{6.6}$MICLD$_{50}$/0.03 ml and titration II had a value of $10^{3.8}$MICLD$_{50}$/0.03 ml. Titration III could not be calculated because of the erratic death pattern of the inoculated mice. All mouse mortality was verified as rabies by the FAT.

Foxes, skunks, dogs and mongoose were given coated SNP in various doses and followed at weekly intervals for rabies serum neutralizing antibody. Two of eight foxes and one of eight skunks receiving 4.0 gm of SNP dispersed in ground meat developed demonstrable rabies antibody. Two of two mongoose receiving 2.0 gm of SNP in meat developed antibody titers while six dogs receiving 2.0 gm or 4.0 gm of SNP either directly into the stomach by gastronomy or fed sausage baits remained serologically negative. Two additional dogs and mongoose fed 0.4 gm of SNP in meat bait also failed to develop demonstrable antibody.

Virus titrations in mice of the coated SNP at weekly intervals after coating indicated a complete loss of viable virus within 3 weeks, which I attributed to the Penford gum. It was subsequently determined that the SMB vaccine virus could be coated onto SNP with uncooked starch in place of the gum, thus precluding the need for the hydroxy ethyl ether derivative. Additional investigation revealed

---

[1] Eastman Kodak Co.
[2] Shinetsu Chemical Co.
[3] Wisconsin Alumni Research Foundation
[4] Penick and Ford, LTD. Cedar Rapids, Iowa

substitution of absolute methyl alcohol for ethyl alcohol had a less deleterious effect on the rabies virus.

A second coating trial was performed whereby 10% ERA-SMB was diluted in a 5% uncooked corn starch solution in buffered saline and coated onto SNP as originally described. The final enteric coat was %5 HMP in equal parts methylene chloride and absolute methyl alcohol. The amount of SMB and SNP were identical to the first trial resulting in the same dosage level.

Virus titration in mice, as described above, gave the following results: a titer of $10^{6.0}$MICLD$_{50}$ prior to coating a titer of $10^{3.5}$MICLD$_{50}$/0.03 ml for titration II while titration III again repeated the erratic death pattern to the mice observed in the first trial. Allowing the crushed SNP to remain overnight at 22°C and 4°C and/or digesting the SNP with pancreatin before titration failed to reverse this phenomenon.

Six skunks and six foxes were fed 2.0 gm of SNP in ground meat baits while eight dogs were fed the same dosage in sausage baits. The two-week bleedings post inoculation of the dogs, foxes and skunks failed to demonstrate antibody. [The serological results on the dogs has yet to be determined.]

Although the results to date are far from encouraging we have confirmed the enteric route as a method of oral rabies vaccination for foxes, skunks and mongoose. The technical difficulties of applying the Wurster air suspension process to the production of a oral rabies vaccine requires further investigation preferable with pharmaceutical consultation. The time period for the first coating could hopefully be shortened by using BHK$_{21}$ tissue culture propagated ERA virus if adherence to the SNP can be accomplished. The inclusion of starch and/or mouse brain to the virus solution in amounts compatible with adherence while being in less concentrated amounts to slow the coating process would be one method of combating the problem if it arises.

The most serious drawback at this point appears to be the deleterious effects to the virus by the organic solvents of the HMP. This can possibly be counteracted by the addition of a gelatin coating prior to the application of the final enteric coat.

The results to date have not lessened our enthusiasm that the application of rabies vaccine onto SNP will be the method most applicable to vaccine-bait incorporation. The major advantage of the SNP would be in the great number of diverse baits that could be used depending on the vector species to be vaccinated. Finally, more meaningful safety testing in non-target species could then be accomplished with the vaccine-bait combination.

References Cited

1. Debbie, J.G. 1974. Use of inoculated eggs as a vehicle for the oral rabies vaccination of red foxes (Vulpes fulva). Inf. Immun. 4:681-683.

2. Ferris, D.H. 1974. TGE virus vaccine. U.S. Patent Office 38223228.

3. Nelson, R.E. and R.L. Linder 1972. Percentage of raccoons and skunks reached by egg baits. J. Wild1. Mgt. 36(4): 1327-1329.

4. Winkler, W.G. and G.M. Baer 1976. Rabies immunization of red foxes (Vulpes fulva) with vaccine in sausage baits. Amer. J. Epidem. 103(4): 408-415.

5. Winkler, W.G., R.G. McLean and J.C. Cowart 1975. Vaccination of foxes against rabies using ingested baits. J. Wild1. Dis. 11: 382-388.

Titration of Rabies Virus in Mice During Enteric Coating Procedure No. 1

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Titer $MICLD_{50}$/0.03 ml
I ERA-SMB[1] in 5% Penford Gum 280	5/5[2]	5/5	5/5	5/5	5/5	4/5	1/5	6.6
II SNP[3] coated with ERA-SMB	5/5	5/5	5/5	1/5	0/5	0/5	0/5	3.8
III SNP coated with ERA-SMB and HMP[4]	1/4	0/4	1/4	1/4	0/4	0/4		-

Titration of Rabies Virus in Mice During Enteric Coating Procedure No. 2

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Titer $MICLD_{50}$/0.03 ml
I ERA-SMB[1] in 5% Penford Gum 280	4/4[2]	4/4	4/4	4/4	4/4	2/4	0/4	6.0
II SNP[3] coated with ERA-SMB	4/4	¾	4/4	0/4	0/4	0/4	0/4	3.5
III SNP coated with ERA-SMB and HMP[4]	1/4	0/4	1/4	0/4	0/4	0/4		-

[1] ERA rabies virus propagated in suckling mouse brain.
[2] Number dead over number inoculated.
[3] Sugar nonpareils.
[4] Hydroxypropyl methylcellulose phthalate.

Presented at:

1) Panel on Viral Diseases
Japanese-U.S. Cooperative Medical Science Program
Sendai, Japan August 25-27, 1976

2) Symposium on Recent advances in Rabies
Atlanta, GA September 7-9, 1976

3) American Public Health Association
Annual Meeting
Miami, FL October 18-22, 1976