Strains of Rabies Virus Available for Preparation of Sylvatic Rabies Vaccines with Special Reference to Vaccines Prepared in Cell Culture

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SUMMARY

Rabies vaccines for use as a preventive in wildlife have not yet been licensed.

There are several vaccines which, based on tests in dogs, merit trial especially in foxes and skunks and these are summarized in a table.

Trials are being conducted in Canada on the effectiveness of several of these vaccines in foxes and skunks.

RÉSUMÉ

Souches de virus rabique disponibles pour la préparation de vaccins destinés aux animaux sauvages et préparés sur cultures cellulaires

On n'a pas encore approuvé de vaccins antirabiques, pour l'immunisation préventive des animaux sauvages.

Des épreuves effectuées chez le chien, ont démontré l'existence de plusieurs vaccins qui méritent un essai, surtout chez le renard et la mouffette; le Tableau I présente un résumé de ces vaccins.

On effectue actuellement des expériences, au Canada, sur l'efficacité de plusieurs de ces vaccins, chez le renard et la mouffette.

INTRODUCTION

Early rabies vaccines were prepared from infected brain or spinal cord of rabbits and larger animals. Later, vaccine prepared from infected duck embryos was introduced. The rabies virus in such vaccines was originally inactivated by desiccation and subsequently by phenol, formalin or beta propiolactone. Inactivated vaccines have been very widely used in man, companion and domestic animals. In animals, vaccines are mostly used pre-exposure as a preventive, and in man after exposure to a rabid animal, although high-risk personnel may receive vaccine pre-exposure.

The first widely used attenuated live virus vaccine for animals was Flury vaccine prepared from chick embryos. This was used originally as the low egg passage (LEP) strain (4). Later, the Flury high passage strain (HEP) was introduced. These vaccines represented the first major attempt to improve the quality of rabies vaccines by reduction of the amount of "foreign" protein present in addition to the viral antigen.

CELL CULTURE VACCINES

Since the early 1960's, following improvements in cell culture techniques, many new vaccines have been prepared in cultures of animal or human cells. These have been used either as attenuated live or inactivated vaccines in animals, or as inactivated vaccines in man. Several reviews and reports cover these various phases in the development of rabies vaccines (see e.g. 3, 5, 6, 7, 9, 11).

The most recent development is the introduction for use in man of human diploid cell vaccines (HDCV) pioneered by the Wistar Institute group (12), as the standard method for pre- and postexposure vaccination. These vaccines will quickly replace animal brain vaccines (e.g. Semple vaccine), duck embryo vaccine (DEV) and other less effective cell culture pre-exposure vaccines.

CURRENT TRIALS IN FOXES AND SKUNKS

There are not yet any formally licensed and approved vaccines for use in wildlife, but there are many readily available vaccines which merit trial in foxes and skunks on the basis of efficacy in dogs (2, 10). Such trials are now in progress in Connaught Laboratories Limited, Willowdale, Ontario under the general supervision of the Rabies Advisory Committee, Ontario Ministry of Natural Resources, with Provincial Lottery funds provided by the Ontario Government.

It is the purpose of this note to present in tabular form (Table I) the various strains of rabies virus which when incorporated in vaccines could be tried for effectiveness in foxes and skunks. Key references are provided in the table. On account of the expense of such a trial, choice has had to be limited to not more than half-a-dozen of the most promising and readily available strains.

Examination of the table reveals that although many vaccines are available, all the rabies virus strains represented are "descended" from one of only three parent strains, the Pasteur original 1882 strain, the Flury strain, and the SAD strain (see e.g. Reference 1).

It has also to be considered that an inactivated vaccine prepared from infected suckling mouse tissues and marketed as "Trimune" is a very potent antigen, and also should be tested in parallel with cell culture vaccines for immunizing potency in foxes (8).

TABLE I

STRAINS OF RABIES VIRUS ADAPTED TO CELL CULTURE: POTENTIAL CANDIDATES FOR USE IN INACTIVATED SYLVATIC VACCINES

Derivation of Strain	Grows in Primary Cells or Cell Line	Currently Available from	Inactivating Agent	References
Section 1. Strains Derived from Original Pasteur Strain (1882) Isolated from Spinal Cord of Rabid Dog and Passed in Rabbits				
1.1 **CVS.** Mouse brain strain of fixed virus. Official challenge strain for vaccine potency (NIH). Used for production of duck embryo vaccine in USA. Derived from Pasteur strain by further passage in mouse brain. CVS-11 strain adapted to cell culture by Kissling	Primary hamster kidney cells, BHK cell line, and human diploid cell lines	Beecham. Rabcine[a] for dogs, and Rabcine-Feline[a] for cats, hamster cell line, inactivated	Phenol	1. Kissling, R.E. Growth of rabies virus in non-nervous tissue culture. Proc. Soc. exp. Biol. Med. 98. 223-225. 1958. 2. Ott, G.L. and B. Heyke. Preliminary trials of a new tissue culture rabies vaccine. Vet. Med. 57: 158-159. 1962. 3. Ott, G.L. and B. Heyke. Propagation of rabies virus; evaluation of a vaccine. Vet. Med. 57: 613-616. 1962.
		Beecham. Rabtect[a] for dogs, hamster cell line, live	None	
1.2 **Paris-Pasteur (PV).** Strain of rabbit-fixed virus. Maintained and distributed by Institut Pasteur, Paris	Primary hamster kidney cell cultures, human diploid cell lines (WI-38 and MRC-5), BHK-21 cell line, primary fetal bovine kidney tissue	Institut Pasteur, Paris, France	Betapropiolactone (BPL); also used in live form	1. Atanasiu, P. M. Ribeiro et H. Tsiang, Vaccins antirabiques de culture cellulaire obtenus avec la souche Pasteur. Résultats de vaccination. Annls Inst. Pasteur, Paris 123: 427-441. 1972. 2. Atanasiu, P., A. Gamet, A. Velch et H. Tsiang. À propos du vaccin antirabique de premier explant sur rein de foetus de veau à usage humain. Symp. Series Immunobiol. Standard. 21: 207-212. 1972. 3. Majer, M., A. Herrmann, J. Hilfenhaus, R. Mauler, H-G. Lehmann, W. Hennessen and E.K. Kuwert. A comparison of the Pasteur and Pitman-Moore strains of rabies for the production of rabies vaccine in human diploid cells. J. Biol. Standard. 5: 249-256. 1977. 4. Atanasiu, P., H. Tsiang, P. Reculard, F. Aguilon, M. Lavergne and Ph. Adamovicz. Zonal centrifuge purification of human rabies vaccine obtained on bovine fetal kidney cells. Biological results. Develop. Biol. Standard. 40: 35-44. 1978.
1.3 **Pitman-Moore or PM Strain.** Maintained in rabbit brain at NIH; distributed in US as official strain for rabbit brain vaccine (PV-11)	BHK-21 cell line		BPL, acetylethylenimine, binary ethylenimine	1. Larghi, O.P., V.O. Savy, A.E. Nebel and A. Rodriguez. Ethylenimine-inactivated rabies vaccine of tissue culture origin. J. clin. Microbiol. 3: 26-33. 1976. 2. Larghi, O.P. and A.E. Nebel. Rabies virus inactivation by binary ethylenimine: new method for inactivated vaccine production. J. clin. Microbiol. 11: 120-122. 1980.
1.4 **PM-HDC.** Human diploid cell strain derived from PV 11 (Pitman-Moore PM) strain by adaptation to human diploid cells (PM/WI-38/1503)	Adapted to growth in WI-38 human diploid fibroblast cells in 1962-1963. Master seed distributed to 3 companies by the Wistar Institute. Adapted also to MRC-5 cells	Wyeth, Philadelphia, Pa. USA (ultrafilter concentrated "split" vaccine)	Tri-N-butyl phosphate (TNP)	1. Wiktor, T.J., M.V. Fernandes and H. Koprowski. Cultivation of rabies virus in human diploid cell strain WI-38. J. Immun. 93: 353-366. 1964. 2. Koprowski, H. Vaccines against rabies: present and future. Pan American Health Organization Scientific Publication No. 147. pp. 488-493. 1967. 3. Wiktor, T.J. E. Gyorgy, H.D. Schlumberger, F. Sokol and H. Koprowski. Antigenic properties of rabies virus components. J. Immun. 110: 269-276. 1973.
		Institut Mérieux, Lyon, France (ultrafilter concentrated "whole virion" vaccine)	BPL	
		Behringwerke, A.G., Marburg/Lahn, West Germany (concentrated and purified by rate zonal centrifugation in a	BPL	

TABLE I — CONTINUED

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Derivation of Strain	Grows in Primary Cells or Cell Line	Currently Available from	Inactivating Agent	References
		sucrose density gradient, before inactivation; "whole virion" vaccine)		4. Majer, M., A. Herrmann, J. Hilfenhaus, R. Mauler, H-G. Lehmann, W. Hennessen and E.K. Kuwert. A comparison of the Pasteur and Pitman-Moore strains of rabies virus for the production of rabies vaccine in human diploid cells. J. Biol. Standard. 5: 249-256. 1977. 5. Plotkin, S.A., T.J. Wiktor, H. Koprowski, E.I. Rosanoff and H. Tint. Immunization schedules for the new human diploid cell vaccines against rabies. Am. J. Epidem. 103: 75-80. 1976. 6. Wiktor, T.J., S.A. Plotkin and H. Koprowski. Development and clinical trials of the new human vaccine of tissue culture (Human Diploid Cell) origin. Develop. Biol. Standard. 40: 3-9. 1978.
	Primary dog kidney cells	Ultrafiltration and column separation	BPL	1. van Wezel, A.L., G. van Steenis, Ch. A. Hannik and H. Cohen. New approach to the production of concentrated and purified inactivated polio and rabies tissue culture vaccines. Develop. Biol. Standard. 41: 159-168. 1978.
1.5 A fixed virus strain from Wistar Institute	NIL line of hamster kidney fibroblasts	Institut Mérieux, Lyon, France "Rabiffa," for veterinary use	BPL	1. Petermann, H.G., R. Lang, R. Branche et J.P. Soulebot. Un nouveau vaccin antirabique préparé avec du virus fixe produit sur culture de cellules et inactivé. Congrès Mondial Vétérinaire, Paris 18: 227-229. 1967.
Section 2. Strains Derived from Flury Strain (1939). Isolated by Intracerebral Injection of Mice with Suspension of Spinal Cord of Girl who Died in Georgia				
2.1 **Flury Low Egg Passage (LEP).** Passed by intracerebral route in one day old chicks and then in 7 day chick embryos	Chick embryo fibroblast cell culture, human diploid cell line, BHK-21 cell line, also pig kidney cell line	Behringwerke. Also produced experimentally in England	LEP was usually used as a modified live vaccine. Acetylethylenimine and BPL have been used to inactivate	1. Leach, C.N. and N.H. Johnson. Human rabies with special reference to virus distribution and titer. Am J. trop. Med. 20: 335-340. 1940. 2. Koprowski, H. and H.R. Cox. Studies on chick embryo adapted rabies virus I. Cultural characteristics and pathogenicity. J. Immun. 60: 533-554. 1948. 3. Johnson, H.N. Experimental and field studies of canine rabies vaccination. *In* Viral and Rickettsial Infections of Man. 3rd Edition. T.M. Rivers and F.L. Horsfall, Eds. p. 407. Philadelphia: Lippincott. 1959. 4. Cabasso, V.J., M.R. Stebbins, A.B. Douglas and G. R. Sharpless. Tissue-culture rabies vaccine (Flury LEP) in dogs. Am. J. vet. Res. 26: 24-32. 1965. 5. Crick, J. and F. Brown. Viral subunits for rabies vaccination. Nature 222: 92. 1969. 6. Barth, R. and O. Jaeger. Vaccination trials with a new thermoinactivated tissue culture rabies vaccine. Berl. Münch. tierärztl. Wschr. 83: 87. 1970. 7. Crick, J. and F. Brown. An inactivated baby hamster kidney cell rabies vaccine for use in dogs and cattle. Res. vet. Sci. 12: 156-161. 1971.

TABLE I — CONTINUED

STRAINS OF RABIES VIRUS ADAPTED TO CELL CULTURE: POTENTIAL CANDIDATES FOR USE IN INACTIVATED SYLVATIC VACCINES

Derivation of Strain	Grows in Primary Cells or Cell Line	Currently Available from	Inactivating Agent	References
				8. Chapman, W.G., I.A. Ramshaw and J. Crick. Inactivated rabies vaccine produced from the Flury LEP strain of virus grown in BHK-21 suspension cells. Am. J. Microbiol. 26: 858-862. 1973.
2.2 **Flury High Egg Passage (HEP).** Further passage of Flury LEP in 7 day chick embryos	Primary chick embryo fibroblasts, dog kidney cell line, and human diploid WI-38 cells	Norden Endurall-R[a] Canine cell line vaccine for dogs and cats	Used as a modified live vaccine, or inactivated by BPL or ultraviolet irradiation	1. Koprowski, H. Biological modification of rabies virus as a result of its adaptation to chicks and chick embryos. Bull. Wld Hlth Org. 10: 709-724. 1954. 2. Wiktor, T.J., M.V. Fernandes and H. Koprowski. Cultivation of rabies virus in human diploid cell strain WI-38. J. Immun. 93: 353-366. 1964. 3. Brown, A.L., E.V. Davis, D.L. Merry and W.H. Beckenhauer. Comparative potency tests on modified live-virus rabies vaccine produced from Flury high egg-passage virus grown on permanent dog kidney cell line. Am. J. vet. Res. 28: 751-759. 1967. 4. Kondo, A., Y. Takashima and M. Suzuki. Inactivated rabies vaccine of chick embryo cell culture origin. Symp. Series Immunobiol. Standard 21: 182-189. 1972.
2.2.1 **HEP-675**	Strain adapted to BKH-21 cell line	Mycofarm BHK-21 cell line	BPL. Also studied in live form	1. Bijlenga, G. et L. Joubert. Possibilité de guérison chez la souris d'une infection très avancée à virus rabique sauvage grâce à la vaccination post-infectieuse. Rev. Inst. Pasteur, Lyon 10: 35-47. 1977. 2. Bijlenga, G. A potency test which simulates natural exposure for measuring post-exposure activity of rabies vaccines. A proposal for preparing a relevant international preparation. Develop. Biol. Standard. 40: 203-208. 1978.
Section 3. Strains Derived from SAD (Street-Alabama-Dufferin) Strain (1953) Isolated from Dog at CDC Montgomery, Alabama and Passed in Mouse Brain				
3.1 **SAD-CL 60**	Adapted to growth in primary hamster kidney cells	Connaught Laboratories Ltd. (for human pre-exposure vaccination)	Formalin, with alum phosphate	1. Fenje, P. Propagation of rabies virus in cultures of hamster kidney cells. Can. J. Microbiol. 6: 479-484. 1960. 2. Fenje, P. A rabies vaccine from hamster kidney tissue cultures: preparation and evaluation in animals. Can. J. Microbiol. 6: 605-609. 1960. 3. Fenje, P. and L. Pinteric. Potentiation of tissue culture rabies vaccines by adjuvants. Am. J. publ. Hlth 56: 2106-2113. 1966. 4. Fenje, P. The status of existing rabies vaccines. *In* International Conference on the Application of Vaccines against Virus, Rickettsial, and Bacterial Diseases of Man. pp. 60-65. Scientific Publication No. 226. Pan American Health Organization. Washington, D.C. 1971.
3.2 **CL 77.** Strain derived by adapting CL-60 to MRC-5 human diploid cells	MRC-5 human diploid cell line. Under trial for human use, both pre-and postexposure	Connaught Laboratories Ltd. (concentrated by ultrafiltration)	BPL	1. Cho, H.C. and P. Fenje. Biological properties of a rabies vaccine produced in the MRC-5 strain of human diploid cells. Can. J. publ. Hlth 70: 60. 1979.

TABLE I — CONTINUED

STRAINS OF RABIES VIRUS ADAPTED TO CELL CULTURE: POTENTIAL CANDIDATES FOR USE IN INACTIVATED SYLVATIC VACCINES

Derivation of Strain	Grows in Primary Cells or Cell Line	Currently Available from	Inactivating Agent	References
3.3 **SAD High Cell Passage**	Canine cell line (Philips Roxane)	Bio-Ceutic. Neurogen-TC[a] and Unirab[a] for dogs	Used live	
		Pitman-Moore Rabvax[a] for dogs	Used live	
3.4 **SAD High Cell Passage**	Bovine-kidney cell line (Pitman-Moore)	Pitman-Moore Rabies vaccine[a] for dogs	Used live	
3.5 **ERA.** Derived from SAD after numerous mouse brain passages. Passaged in mouse brain, hamster kidney cell culture, chick embryo (7 day old) by yolk sac, and pig kidney (porcine) cell culture	Commercial ERA produced in pig kidney cell cultures	1. Connaught Laboratories Ltd. 2. Jensen-Salsbery (USA) ERA Strain Rabies vaccine[a] Used fog dogs, cattle, horses, sheep, goats	Used live	1. Abelseth, M.K. Propagation of rabies virus in pig kidney cell cultures. Can. vet. J. 5: 84-87. 1964. 2. Abelseth, M.K. An attenuated rabies vaccine for domestic animals produced in tissue culture. Can. vet. J. 5: 279-286. 1964. 3. Abelseth, M.K. An attenuated rabies vaccine for domestic animals produced in tissue culture. Symp. Series Immunobiol. Standard. 1: 367-375. 1965. 4. Lawson, K.F., V.C.R. Walker and J.F. Crawley. ERA strain rabies vaccine: Duration of immunity in cattle, dogs and cats. Vet. Met. 62: 1073-1074. 1967. 5. Abelseth, M.K. and J.F. Crawley. ERA rabies vaccine. World Vet. Cong., Paris 18: 235. 1967.
3.5.1 **ERA** (Baer)	Baer's ERA produced in BHK-21 cell line	Used experimentally in the U.S.	Used live	1. Baer, G.M. Wildlife vaccination. *In* The Natural History of Rabies. Vol. II. pp. 261-266. New York: Academic Press. 1975.

REFERENCES

1. CLARK, H.F. and T.J. WIKTOR. Rabies virus. *In* Strains of Human Viruses. M. Majer and S.A. Plotkin, Eds. Basel: S. Karger. 1972.
2. COMPENDIUM OF ANIMAL RABIES VACCINES. Bureau of Epidemiology, CDC, Atlanta, Georgia. CDC Veterinary Public Health Notes. January 1980.
3. CRICK, J. The vaccination of man and other animals against rabies. Postgrad. med. J. 49: 551-564. 1973.
4. KOPROWSKI, H. and H.R. COX. Studies on chick-embryo adapted rabies virus: 1. Cultural characteristics and pathogenicity. J. Immun. 60: 533-554. 1948.
5. PLOTKIN, S.A. Rabies vaccine prepared in human cell cultures: progress and perspectives. Rev. infect. Dis. 2: 433-448. 1980.
6. SIKES, R.K. Rabies vaccines. Archs envir. Hlth 19: 862-867. 1969.
7. SIKES, R.K. Canine and feline vaccines—past and present. *In* The Natural History of Rabies. Vol. II. G.M. Baer, Ed. New York: Academic Press. 1975.
8. SIKES, R.K. and O.P. LARGHI. Purified rabies vaccine: development and comparison of potency and safety with two human rabies vaccines. J. Immun. 99: 545-553. 1967.
9. VAN ROOYEN, C.E. and A.J. RHODES. Virus Diseases of Man. New York: Thomas Nelson and Sons. 1948.
10. VETERINARY BIOLOGICS STAFF, AGRICULTURE CANADA. Animal rabies vaccines marketed in Canada. Can. vet. J. 21: 241. 1980.
11. WHO EXPERT COMMITTEE ON RABIES. Sixth Report. WHO Tech. Rep. Series No. 523. Geneva, Switzerland: World Health Organization. 1973.
12. WIKTOR, T.J., M.V. FERNANDES and H. KOPROWSKI. Cultivation of rabies virus in human diploid cell strain WI-38. J. Immun. 93: 353-366. 1964.