

STABILITY OF RABIES SUCKLING MOUSE BRAIN VACCINE STORED AT DIFFERENT TEMPERATURES¹

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INTRODUCTION

Rabies in man and animals continues to be a serious problem for public health and the livestock economy in most countries of Latin America and the Caribbean (1). Between 1980 and 1985 this area had a total of 1,744 registered rabies cases in humans, 83,858 in dogs, 6,876 in cats, and 28,800 in cattle—although a considerable share of the bovine cases are usually not reported (2).

According to data submitted by the countries of Latin America and the Caribbean to the Pan American Zoonoses Center in Buenos Aires, Argentina, during 1985 these countries prepared more than 5.2 million doses of suckling mouse brain (SMB) vaccine at 19 official production laboratories for the immunization of three million people, this being about the number requiring rabies treatment annually. In addition, in that same year official and private laboratories produced around 25 million doses of antirabies vaccine for veterinary use, 87% of which were SMB vaccine (3).

Because mass vaccination of dogs and timely treatment of exposed people constitute the most effective rabies control measures (4, 5), it is essential to have vaccines that are potent, safe, stable, and economical. The SMB rabies vaccine for human and animal use is inexpensive to produce and is known to have good antigenic potency (6, 7). However, the literature does not mention any systematic studies on its stability. We therefore conducted the study reported here, which was directed at determining the SMB vaccine's ability to retain its immunizing power at different temperatures and for different periods of time.

MATERIALS AND METHODS

Liquid SMB Vaccines

Six lots of SMB vaccine for human use (SMB-H) and six for canine use (SMB-C) were prepared at our laboratory according to the technique described by

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Fuenzalida (7). The antigenic values of these lots were determined by the potency test of the United States National Institutes of Health (NIH test) (8) and were found to be within the most commonly observed ranges. In addition, one lot of SMB vaccine for bovine use (SMB-B) was prepared according to the technique described by Díaz and Lombardo (9).

Lyophilized SMB Vaccines

Twelve additional lots of SMB test vaccine were prepared—six for human and six for canine use. Half of these were lyophilized in a Virtis apparatus (made by the Virtis Co., Inc., Gardiner, NY, USA) so as to have residual moisture levels between 2% and 3%. The lyophilization cycle was as follows: Flasks, 50 per lot and each with 2 ml of product, were placed on shelves in the lyophilization chamber until they reached -50°C , after which they were dried in a vacuum atmosphere (at a pressure of five microns of mercury) for 72 hours and sealed under vacuum. The final temperature of the product was 18°C .

The other six lots were prepared so that their residual moisture levels were between 5% and 7%. The lyophilization stabilizer used in both cases was 7.5% sucrose and 0.5% gelatin.

In addition, we prepared a lot of lyophilized SMB-B vaccine with a 2% residual moisture level that was reconstituted with a diluent containing 2% aluminum hydroxide (9, 10).

The residual moisture levels of the various lots were determined by the Karl Fisher method (11).

Stability Tests

Samples of each vaccine lot were stored at three different temperatures: 4°C , 25°C , and 37°C ; and the antigenic potency of each was determined periodically using the NIH test (8). For purposes of this test, CF-1 mice weighing 10 to 14 g were employed; the reference vaccine used was CPZ-10, which had a required minimum potency coefficient of 0.2. All of the vaccines were tested as a group at each of the scheduled times.

A regression analysis for all the vaccine lots prepared was performed using the least squares method (12).

RESULTS

Liquid SMB Vaccines

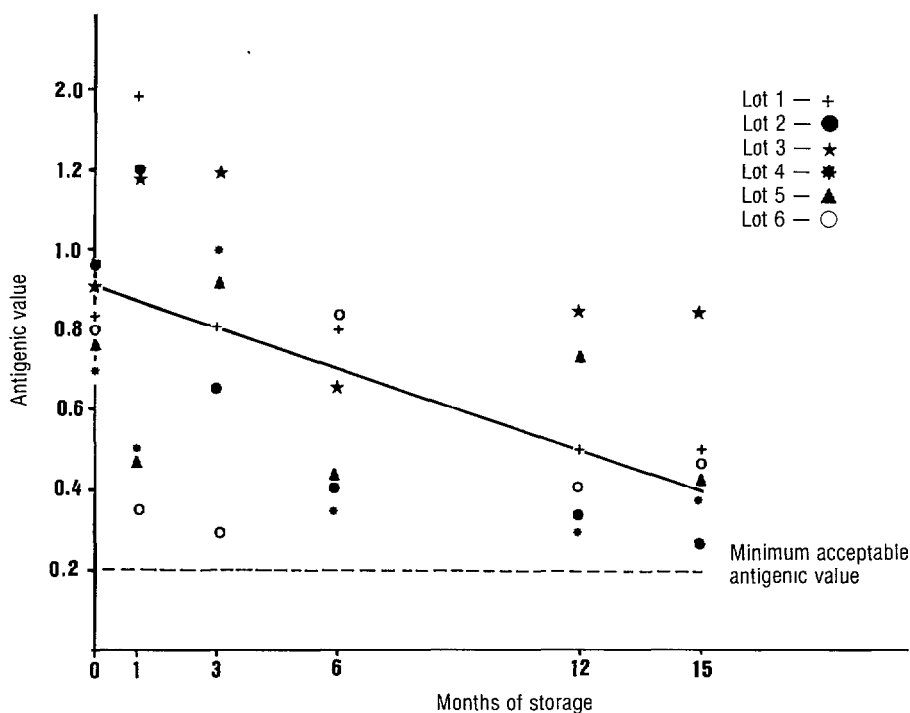
The initial antigenic values of the liquid SMB-H vaccine lots were as follows: lot 1 = 0.8; lot 2 = 0.95, lot 3 = 0.9, lot 4 = 0.7, lot 5 = 0.8, and lot 6 = 0.8. For the SMB-C vaccine lots the values were: lot 1 = 5.0, lot 2 = 4.6, lot 3 = 6.7, lot 4 = 1.5, lot 5 = 1.5, and lot 6 = 1.5. Finally, the value of the one lot of SMB-B vaccine was 5.3.

Table 1 shows the stability of the different types of liquid SMB vaccine when stored at different temperatures. The six lots of SMB-H vaccine, which had an average initial antigenic value of 0.8, all maintained an antigenic value exceeding 0.2 for 15 months when stored at 4°C , their final average antigenic value being 0.5. All six also kept above the minimum value of 0.2 for 30 days when stored at 25°C , the final average antigenic value at 30 days being 0.4; and four of the six lots remained above the

TABLE 1. The numbers of lots of different kinds of liquid SMB antirabies vaccine meeting minimum acceptable potency requirements after storage for different lengths of time at different temperatures. The numbers of lots tested were six of SMB-H, six of SMB-C, and one of SMB-B.

Storage time (months)	Liquid SMB vaccine lots of different types stored at the temperatures shown								
	For humans (SMB-H)			For canine animals (SMB-C)			For bovine animals (SMB-B)		
	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
1	6	6	4	6	6	1	1	1	1
3	6	0	0	6	1	0	1	1	1
6	6	0	0	6	0	0	1	1	0
12	6	0	0	6	0	0	1	1	0
15	6	0	0	6	0	0	1	1	0

FIGURE 1. Observed changes in the potency of the six lots of liquid SMB antirabies vaccine for humans during the fifteen-month storage period. Linear regression: $y = 0.919 - 0.032x$.



minimum value for 30 days when stored at 37°C, the final average antigenic value of these four at 30 days being 0.3.

The six lots of SMB-C vaccine, which had an average initial antigenic value of 4.5, also stayed above the 0.2 minimum value when stored for 15 months at 4°C, their final average antigenic value being 1.5. Likewise, all six lots maintained antigenic values above the minimum after 30 days of storage at 25°C; and one lot did so after three months of storage at 37°C—its initial antigenic value being 3.5 and its final value after three months being 0.4.

Figure 1 shows how the potency of the six lots of SMB-H vaccine declined during the study period. The linear regression calculated by the least squares method had a slope of -0.032 , indicating an estimated average potency loss of 41.7% after 12 months of storage at 4°C and of 52.3% after 15 months of storage at this temperature.

Figure 2 provides a similar diagram for the six lots of SMB-C vaccine. In this case the linear regression was found to have a slope of -0.201 , indicating an estimated average potency loss of 47.7% at 12 months of storage and of 59.6% at the end of 15 months.

In three of the 12 lots of liquid vaccine studied (lots 5 and 6 of SMB-H and lot 2 of SMB-C) the vaccine's potency appeared to decline relatively slowly—the average decline relative to initial values being only 21% at both 12 and 15 months of storage at 4°C.

As Tables 1 and 2 show, the single lot of liquid SMB-B maintained a potency above the minimum level when stored for 15 months at 4°C, its initial and final antigenic values being 5.3 and 1.2. It also retained above-minimum potency for 15 months at 25°C (the final antigenic value being 0.3), and for three months at 37°C (the final antigenic value being 1.6).

Lyophilized SMB Vaccines

The initial antigenic values of the six lyophilized lots of SMB-H/SMB-C vaccine with residual moisture levels of 2–3% were as shown:

Lot No.	Initial antigenic values of six lots of lyophilized SMB vaccines	
	For humans (SMB-H)	For canine animals (SMB-C)
1	1.5	2.9
2	2.3	3.8
3	1.4	1.0

In contrast, the initial antigenic values of the lyophilized lots of SMB-H/SMB-C vaccine with residual moisture values of 5–7% were much lower:

Lot No.	Initial antigenic value
1	0.9
2	0.6
3	0.4
4	0.4
5	0.3
6	0.5

The initial antigenic value of the lyophilized SMB-B vaccine lot was 8.9. This vaccine retained acceptable potency when stored for 15 months at the three test temperatures (see Table 2).

FIGURE 2. Observed changes in the potency of the six lots of liquid SMB antirabies vaccine for dogs during the fifteen-month storage period. Linear regression: $y = 5.053 - 0.201x$.

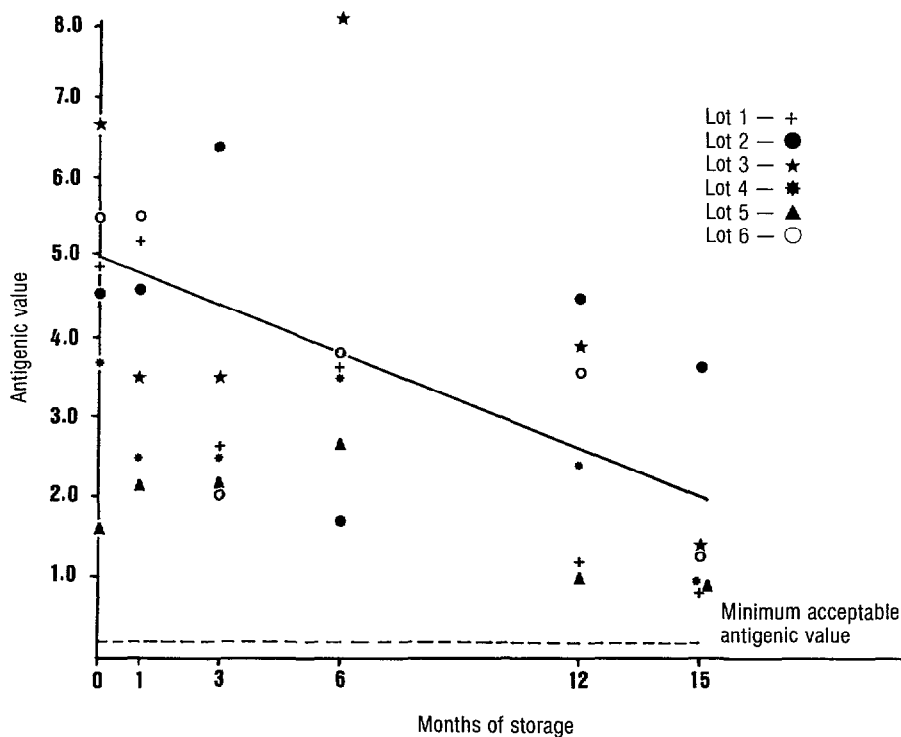


TABLE 2. Antigenic values of the SMB antirabies vaccine for bovine use (liquid and lyophilized), by storage times and temperatures.^a

Storage time (months)	Antigenic values of SMB vaccine for bovine use					
	Liquid			Lyophilized		
	4°C	25°C	37°C	4°C	25°C	37°C
1	4.1	2.1	2.7	14.0	5.7	6.1
3	3.0	4.6	1.6	5.5	7.8	10.0
6	6.8	0.9	0	11.0	8.8	5.8
12	0.6	0.4	0	5.3	6.2	3.2
15	1.2	0.3	0	6.6	5.2	3.6

^a Minimum potency requirement = 0.2.

Table 3 indicates the stability of the 12 lots of lyophilized SMB-H/SMB-C vaccines, six with a residual moisture level of 2–3% and six with a residual level of 5–7%. The data show that all the vaccine lots with a residual moisture level of 2–3% maintained an acceptable potency for storage periods ranging up to 24 months at all three storage temperatures (4°C, 25°C, and 37°C). In contrast, the vaccine lots with 5–7% residual moisture levels only retained acceptable potency during the 24 month study period when they were stored at 4°C. Both the initial and subsequent antigenic values obtained for these lots were lower than the values obtained for the lots with lower moisture levels.

DISCUSSION AND CONCLUSIONS

The results of this study show that the SMB vaccine—in its human, canine, and bovine formulations—retains its immunizing power for prolonged periods of time.

In general, the rabies situation in developing countries of the Americas and elsewhere makes it necessary to have rabies vaccines that are safe, effective, economical, and stable (13). What makes this particularly important is the fact that some 94.2% of all rabies treatments are administered in tropical regions (14).

SMB rabies vaccine is one of the more widely used types because it induces a rapid immunologic response and confers protection against rabies virus infection in both man and animals (10, 15–19).

Nicholas et al. (20) studied the stability of two batches of liquid diploid-cell rabies vaccine for human use at 4°C. They observed that the initial antigenic values (as indicated by the NIH test) of 13.2 and 18.5 fell to 2.9 and 7.2, respectively, after 12 months. In this same vein, Larghi et al. (21) studied the

TABLE 3. Test results obtained with the 12 lots of lyophilized SMB-H/SMB-C vaccines, six with 2–3% residual moisture and six with 5–7%, after storage for the indicated times at 4°C, 25°C, and 37°C. The table shows the number of lots with at least the required minimum potency (0.2) and the average antigenic potency of those lots.

Storage time (months)	No. of lots with indicated residual moisture levels having acceptable potency (average antigenic values in parentheses) after storage at temperatures shown					
	2–3% moisture level (6 lots)			5–7% moisture level (6 lots)		
	4°C	25°C	37°C	4°C	25°C	37°C
1	6 (3.2)	6 (2.2)	6 (1.8)	6 (0.5)	0 (<0.2)	0 (<0.2)
3	6 (3.2)	6 (2.2)	6 (0.6)	6 (0.8)	0	0
6	6 (2.9)	6 (2.9)	6 (1.5)	6 (1.1)	0	0
12	6 (2.7)	6 (3.5)	6 (1.1)	6 (1.6)	0	0
24	6 (3.9)	6 (1.6)	6 (2.0)	6 (0.6)	0	0

stability of a test lot of rabies vaccine for veterinary use produced in BHK-21 cells. They found that the antigenic value fell from 3.9 to 2.3 after 12 months of storage at 4°C. The observed reduction in antigenic potency of the 12 lots of liquid SMB-H and SMB-C vaccines stored at 4°C and tested in the present study was similar to that observed in the cell-culture vaccines cited.

These data indicate that the potency of inactive liquid rabies vaccines tends to diminish over the course of their useful life. Nevertheless, P. Précausta (22), in studying the stability of two batches of vaccine prepared in NIL-2 cells and stored for 12 months at 4°C, did not observe any marked change in their antigenic potency, suggesting that this type of vaccine may be more stable. It should be noted, however, that estimates of rabies vaccine potency based on the NIH test are subject to considerable variation (23, 24), and so it is not wise to generalize about the stability of a particular type of vaccine on the basis of studies involving only a small number of lots. The apparent differences in the performance of three of the 12 lots tested in the study reported here could be due to variations of this kind.

Verification that the liquid SMB vaccines for human and animal use keep their antigenic capacity when stored for 15 months at the recommended temperature (4°C) is important, because in Latin America and the Caribbean there is no uniform standard by which to determine the expiry period, which ranges from 6 to 12 months in all countries except Colombia, where it is 24 months. The results of this study suggest that a one-year expiry period should be appropriate, since after 12 months at 4°C the antigenic values of the SMB-H vaccines tested were observed to fall by 16% to 65.5%, and after 15 months by 18% to 82%. Regarding the SMB-C vaccine

lots, their observed potency fell by 21% to 70% after 12 months and by 24% to 87% after 15 months. It has been demonstrated elsewhere that SMB-C vaccine with an antigenic value of 0.3 protected 100% of a group of dogs challenged one year after vaccination and 80% of a group challenged three years after vaccination (17). Adopting a twelve-month expiry period would avoid the need to discard vaccine lots of verified quality simply because they had exceeded the limits of a briefer expiry period.

In its thirty-first report, the WHO Expert Committee on Biological Standardization recommended that the expiry dates of rabies vaccines be based on the last satisfactory test of activity (25). However, because of the gradual fall in the potency of liquid rabies vaccines over time, it would seem appropriate that the expiry date of SMB vaccine be set at the moment of its production. This is the same criterion stipulated by the United States Code of Federal Regulations (CFR) for the expiry periods of biologicals licensed in that country (26).

If rabies vaccines are lyophilized, their validity period can be extended from 18 to 24 months (25). The stability of such lyophilized vaccines is evaluated mainly by tests of their antigenic potency and residual moisture (27).

In the study reported here, the observed stability of the lyophilized SMB vaccine lots with a 2–3% residual moisture level was comparable to that observed by Roumiantzetz et al. (28), who studied 127 lots of lyophilized vaccines prepared in human diploid cells,

and more recently by Fournier et al. (29), who demonstrated that six lots of purified vaccine obtained from a VERO cell line maintained their potency for 24 months at 4°C and for more than six months at 37°C.

Regarding the lyophilized SMB-C and SMB-B vaccines, our results indicate that these were as stable as vaccines prepared by other workers in NIL-2 cells (22) and BHK-21 cells (30, 31).

The WHO Expert Committee on Biological Standardization has recommended that lyophilized rabies vaccines contain no more than 1% residual moisture (25), and the CFR cites the same value except for a few biologicals such as BCG vaccine and certain viral vaccines (32). However, the nature of some lyophilized biologicals and the additives they contain can interfere with maintenance of 1% residual moisture. Hence, each producer should determine the most satisfactory level of residual moisture that will allow the product to retain its stability throughout the expiry period (27). Thus, for example, in the case of the SMB-H and SMB-C vaccines tested in our study, the six lots with 2–3% residual moisture kept their antigenic potency for 24 months when stored at 4°C, 25°C, and 37°C.

It should be noted that Majer et al. (33) studied the stability of five lots of lyophilized rabies vaccine with 2.6–3.3% residual moisture that had been prepared in human diploid cells. All these lots retained acceptable levels of potency after incubation for four weeks at 37°C, and one lot did so for 12 months at 4°C and 22°C and for six months at 37°C. The four other lots were not tested at six and 12 months.

In our study, the lyophilized SMB-H and SMB-C vaccines with residual moisture levels over 5% kept their antigenicity only when they were stored at 4°C.

Regarding stabilizers, although sucrose and gelatin have been used as stabilizers for different types of rabies vaccines (34, 35), studies have yet to be done on the composition of the most convenient innocuous stabilizer to use in lyophilizing SMB vaccine for human use.

Finally, the good stability of SMB vaccine should not lead users to forget its optimum preservation temperature (4°C–8°C) and thus run the risk that the vaccine's potency will decline below acceptable levels during storage or during use under field conditions.

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SUMMARY

Considering that every year some 300 people die of rabies in Latin America and the Caribbean, it is important for the Region to have rabies vaccines that are potent, innocuous, stable, and inexpensive. The type of rabies vaccine most widely used for both human and animal purposes at present is suckling mouse brain (SMB) vaccine.

To demonstrate the stability of SMB vaccine at different temperatures, test lots of liquid and lyophilized

REFERENCES

SMB vaccine were prepared for human and canine use. Samples were then stored at 4°C, 25°C, and 37°C; and their antigenic potency was checked periodically by the NIH test.

The average antigenic potency of six test lots of liquid SMB vaccine prepared for human use declined by some 52.3%, from an initial average level of 0.8, when stored at 4°C over 15 months. However, all the lots met the minimum potency requirement established by the NIH test at the end of 15 months at 4°C. All six also retained acceptable potency for 30 days when stored at 25°C, and four did so for 30 days when stored at 37°C. Similar patterns were exhibited by lots of liquid vaccine prepared for canine and bovine use.

Regarding the lyophilized SMB vaccines prepared for human and canine use, all of the test lots with a residual moisture level of 2–3% retained an adequate potency for 24 months at all three storage temperatures (4°C, 25°C, and 37°C). In contrast, those with residual moisture levels of 5–7% only retained an adequate potency if they were stored at 4°C.

Overall, these results indicate it is appropriate to establish a one-year expiry period, starting from the date of manufacture, for liquid SMB rabies vaccines stored at 4°C. For lyophilized SMB rabies vaccines with appropriately low residual moisture levels (2–3%), it would seem reasonable to extend the length of the expiry period from 18 to 24 months.

- 1 Honigman, M. N. *La rabia en las Américas, 1970–1979: Análisis y comentarios*. Publicación Especial 3. Centro Panamericano de Zoonosis, OPS/OMS; Ramos Mejía, Argentina, 1981.
- 2 Centro Panamericano de Zoonosis (OPS/OMS). *Vigilancia Epidemiológica de la Rabia en las Américas*. Vol. 12, nos. 7–12, Martínez, 1986.
- 3 Centro Panamericano de Zoonosis (CEPANZO, OPS/OMS). Encuesta sobre laboratorios productores de vacunas antirrábicas. (Unpublished report.) Buenos Aires, 1986.
- 4 World Health Organization. *WHO Expert Committee on Rabies: Seventh Report*. WHO Technical Report Series, No. 523. Geneva, 1984.
- 5 Larghi, O. P. Improvement of Post- and Pre-exposure Immunization in Latin America. In: E. K. Kuwert, T. J. Wiktor, and H. Koprowski (eds.). *Cell Culture Rabies Vaccines and Their Protective Effect in Man*. International Green Cross, Geneva, 1981; pp. 283–287.
- 6 Nicholson, K. G. Improving Rabies Post-exposure Treatment in Developing Countries: Some Suggestions. In: E. K. Kuwert, T. J. Wiktor, and H. Koprowski (eds.). *Cell Culture Rabies Vaccines and Their Protective Effect in Man*. International Green Cross, Geneva, 1981; pp. 288–293.
- 7 Fuenzalida, E., and R. Palacios. Un método mejorado para la preparación de la vacuna antirrábica. *Boletín del Instituto Bacteriológico de Chile* 8(1–4):3–10, 1950.
- 8 Seligman, E. B. Prueba da potencia NIH. In: M. M. Kaplan and H. Koprowski (eds.). *La rabia: Técnicas de laboratorio (3a ed.)*. World Health Organization, Geneva, 1976; pp. 294–302.
- 9 Díaz, A. M. O., and R. A. Lombardo. Inmunización de terneros con vacuna antirrábica de cerebro de ratón lactante. *Revista de la Asociación Argentina de Microbiología* 13(2):45–48, 1981.
- 10 Fuenzalida, E., A. M. O. Díaz, and S. Riven-son. Vacuna antirrábica de cerebro de ratón lactante suplementada con adyuvante: Su aplicación en bovinos. *Revista de la Asociación Argentina de Microbiología* 10(1):47–53, 1973.

- 11 United States Pharmacopeial Convention. *The United States Pharmacopeia, XIX Revision*. Rockville, 1975; pp. 668-669.
- 12 Dixon, W. S., and F. J. Massey. *Introducción al análisis estadístico*. McGraw-Hill, New York, 1980.
- 13 World Health Organization. World Survey of Rabies XXII (for Years 1984-85). Document WHO/Rabies 87/198. Geneva, 1987.
- 14 Acha, P. N., and P. V. Arámbulo III. Rabies in the Tropics: History and Current Status. In: E. Kuwert (ed.). *Rabies in the Tropics*. Springer Verlag, Berlin, 1984.
- 15 Held, J. R., E. Fuenzalida, H. López Adaros, J. C. Arrossi, N. O. R. Poles, and A. Scivetti. Inmunización humana con vacuna antirrábica de cerebro de ratón lactante. *Bol Of Sanit Panam* 72(6):565-575, 1972.
- 16 Díaz, A. M. O., G. González Resigno, A. Fernández Munilla, O. P. Larghi, N. Marchevsky, and J. C. Arrossi. Vacuna antirrábica de cerebro de ratón lactante: Esquemas reducidos de inmunización post-exposición. *Revista de la Asociación Argentina de Microbiología* 11(1):42-44, 1979.
- 17 Sikes, R. K., G. V. Peacock, P. N. Acha, R. J. Arko, and R. Dierks. Rabies vaccine: Duration of immunity study in dogs. *J Am Vet Med Assoc* 159:1491-1499, 1971.
- 18 Serufo, J. C., M. Gonçalves de Lima, Y. Hayashi, J. A. Montañó, E. Mora, and A. J. Belotto. Reduced schedule for the prophylactic treatment of rabies in man with the suckling mouse brain vaccine. *Arquivos de Biología y Tecnología* 28(2):227-243, 1985.
- 19 Schlogel, F., T. M. Monteciro, E. M. Wartelsteiner, and V. M. Kurowski. Canine and feline rabies in the state of Paraná (Brasil). *Arquivos de Biología y Tecnología* 28(2):265-276, 1985.
- 20 Nicolas, A. J., J. Patet, J. C. Vincent-Falquet, R. Branche, P. Delati, B. Montagnon, L. Peyron, and J. P. Soulebot. Production of inactivated rabies vaccine for human use on WI38 diploid cells: Results of potency test, stability of vaccine in liquid and lyophilized forms. *Dev Biol Stand* 40:17-24, 1978.
- 21 Larghi, O. P., V. Savy, A. E. Nebel, and A. Rodríguez. Ethylenimine-inactivated rabies vaccine of tissue culture origin. *J Clin Microbiol* 3(1):26-33, 1976.
- 22 Précausta, P. Vaccine antirabique inactivé a usage vétérinaire préparé a partir de culture cellulaire. *Symposia Series in Immunobiological Standardization* 21:162-178, 1974.
- 23 Wiktor, T. J., P. Atanasiu, M. Bahmanyar, K. Bogel, J. H. Cox, A. M. Díaz, E. A. Fitzgerald, E. Kuwert, R. Netter, M. Selimov, G. Turner, and G. Van Steenis. Comparison studies on potency tests for rabies vaccines. *Dev Biol Stand* 40:171-178, 1978.
- 24 Barth, R., E. Grob-Albenhausen, O. Jaeger, and L. Milcke. The antibody-binding test, a useful method for quantitative determination of inactivated rabies virus antigen. *J Biol Stand* 9:81-89, 1981.
- 25 World Health Organization. *WHO Expert Committee on Biological Standardization: Thirty-first Report*. WHO Technical Report Series, No. 658. Geneva, 1981.
- 26 United States. *U.S. Code of Federal Regulations: Dating Periods for Licensed Biological Products*. Document 21 CFR 610.53, Washington, D.C., 1985; pp. 56-65.
- 27 Seligman, E. B. Review of United States requirements for residual moisture in drugs. *Dev Biol Stand* 36:175-179, 1977.
- 28 Roumiantzeff, M., N. Ajjan, R. Branche, P. Fournier, B. Montagnon, P. Trotemann, and J. C. Vincent-Falquet. Rabies Vaccine Produced in Cell Culture: Production, Control and Clinical Results. In: E. Kurstak (ed.). *Applied Virology*. Academic Press, New York, 1984; pp. 241-296.
- 29 Fournier, P., B. Montagnon, J. C. Vincent-Falquet, N. Ajjan, J. Drucker, and M. Roumiantzeff. A New Vaccine Produced from Rabies Virus Cultivated on VERO Cells. In: I. Vodopija, K. G. Nicholson, S. Smerdel, and U. Bijok (eds.). *Improvements in Rabies Post-Exposure Treatment*. Zagreb Institute of Public Health, Zagreb, 1985.
- 30 Larghi, O. P., and A. E. Nebel. Rabies virus inactivation by binary ethylenimine: New method for inactivated vaccine production. *J Clin Microbiol* 11(2):120-122, 1980.
- 31 Larghi, O. P., and A. E. Nebel. Duration of immunity afforded to cattle by a binary ethylenimine-inactivated rabies vaccine. *Zentralblatt für Veterinärmedizin* 32:609-615, 1985.

- 32 United States. *U.S. Code of Federal Regulations: Test for Residual Moisture*. Document 21 CFR 610.13(a), Washington, D.C., 1985; p. 49.
- 33 Majer, M., A. Herrmann, J. Hilfenhaus, E. Reichert, R. Mauler, and W. Hennessen. Freeze-drying of a purified human diploid cell rabies vaccine. *Dev Biol Stand* 36:285-289, 1977.
- 34 Hoskins, J. M. Vacuna de embrión de pato. In: M. M. Kaplan and H. Koprowski (eds.). *La rabia: Técnicas de laboratorio (3a ed.)*. World Health Organization, Geneva, 1976; pp. 256-269.
- 35 Karakujumcan, M. K., B. M. Patriz, and D. V. Soloviev. Vacuna de encéfalo de rata lactante. In: M. M. Kaplan and H. Koprowski (eds.). *La rabia: Técnicas de laboratorio (3a ed.)*. World Health Organization, Geneva, 1976; pp. 226-228.

Immunization Awareness Week Designated in U.S.

The United States Senate passed a resolution introduced by Sen. John Melcher, Chairman of the Senate Special Committee on Aging, that would permanently designate the last week of October as "National Adult Immunization Awareness Week." The resolution has also been introduced into the House of Representatives, where it is expected to pass.

Sen. Melcher pointed out that tens of thousands of adults, most of them elderly, die each year from diseases that could have been prevented by readily available vaccines, and that the need for adult immunization has been largely neglected. Only one in eight adults in the U.S. is protected against flu, pneumonia, hepatitis B, measles, diphtheria, rubella, or tetanus. Fewer than 10% of the elderly are immunized against pneumococcal pneumonia, which is the sixth leading cause of death in the United States. Influenza, another killer, strikes hardest at the elderly and chronically ill. Each of the 16 major flu epidemics in the past 30 years has claimed the lives of at least 10,000 people, many of whom could have been saved by immunization.

Source: Senate Special Committee on Aging, News Release, June 8, 1988.