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Neurological disease in man following administration of suckling mouse brain antirabies vaccine*

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In Latin America, suckling mouse brain (SMB) vaccine has become the most commonly used vaccine for immunization of both man and animals against rabies. This vaccine is highly immunogenic, is relatively economical and easy to produce, and is believed to be free of the immunoencephalitogenic factor. From 1964 to the end of 1969, there were 40 reported cases of neurological disease following administration of SMB vaccine, 32 of which met the criteria for inclusion in this report. These 32 cases occurred in 8 different countries. In contrast to neurological disease following the administration of other types of nervous tissue vaccine, the majority of the cases following vaccination with SMB vaccine had a Guillain–Barré-type syndrome with peripheral nervous system involvement and a higher case-fatality rate. The causative agent has not been demonstrated. Modifications in the production and handling of the vaccine may be producing changes that are responsible.

INTRODUCTION

Post-exposure immunoprophylaxis of man against rabies has long been the physician's dilemma because of the well-known risk of a neuroparalytic accident resulting from the administration of the vaccine. This type of reaction is generally considered to be allergic in nature and provoked by the presence of central nervous system myelin in vaccines prepared from the brains and/or spinal cords of infected animals. In the past, the animals most commonly used were adult rabbits, sheep, or goats. In an effort to reduce or eliminate this risk, various vaccines have been developed in which the virus is grown in embryonated eggs that are free, or nearly free, of myelin.

Fuenzalida & Palacios (1955) in Chile reported the development of an antirabies vaccine produced in suckling mice. Their primary reason for using suckling mice, which are highly susceptible to rabies virus, was to produce a vaccine with a very high titre. The mice are inoculated when they are 3–5 days old and their brains are harvested when they are 7–9 days old. Myelin has not formed in the brains of mice at that age, and the vaccine is believed to be free of the immunoencephalitogenic factor, which is an additional benefit. Because of this, and because this vaccine is highly immunogenic and is relatively economical and easy to produce, it has become the most commonly used vaccine in Latin America for the vaccination of both man and animals. Nearly all vaccines produced in suckling mouse brains are called Fuenzalida vaccines, but in reality there are several different suckling mouse brain (SMB) vaccines. A 0.03-ml quantity of the vaccine for use in man as originally described and currently produced in many places contains:

supernatant from a 1% suspension of brain tissue,

$10^5 MLD_{50}$ of rabies virus, inactivated by ultraviolet light,

double-distilled water,

phenol 1 : 1 000 as a preservative,

and thiomersal 1 : 10 000 as a preservative.

Some vaccine producers have somewhat empirically modified the vaccine in different ways or have omitted some steps. For example, 0.5% brain supernatant is used instead of 1%, or β-propiolactone (2-oxetanone) or phenol may be used to inactivate the virus instead of UV light: sometimes diluents other than distilled water are employed. Some producers do not centrifuge the brain suspension.

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The first reported neuroparalytic accident following the use of SMB vaccine occurred in a patient in Venezuela (Lopez Adaros, 1967). Further investigation revealed that this case was somewhat uncharacteristic of neuroparalytic accidents following vaccination with other types of nerve-tissue vaccine in that the clinical syndrome concerned the peripheral rather than the central nervous system. In Venezuela, a programme of surveillance was initiated to detect the occurrence of such cases. It was found that one case had occurred in 1966; 14 cases were reported in 1967 and 3 in 1968. The most striking feature of these cases was that most of them involved the peripheral nervous system. Of the 18 cases reported during these 3 years in Venezuela, one was a fatal encephalitis, which could possibly have been caused by a rabies infection, and two were peripheral in nature but were mild, not showing sufficient signs to warrant their inclusion in this study. Of the remaining 15 cases, 11 could be classified as Guillain–Barré syndrome.

Of the 15 cases, 1 occurred in 1966, 11 in 1967, and 3 in 1968 (Table 1). Vaccines produced by two different manufacturers were involved. After the first cases occurred, vaccine production was terminated by producer A and the same type of vaccine was imported from producer B, located in another country nearly 5 000 km away. After the occurrence of the cases, the various lots of vaccine involved were tested for safety, purity, and sterility; and the only significant finding was the presence of minute amounts of active rabies virus in vaccine lot no. 46. Of the four cases of disease that followed vaccination with this lot, only one patient died; and no rabies virus could be isolated from his brain. About 500 patients vaccinated with lot no. 46 were kept under surveillance for more than a year, and there was no evidence that rabies occurred in any of them. No rabies virus could be isolated from any of the other lots of vaccine.

Table 1. Number of cases of neurological disease in man following antirabies prophylaxis with SMB vaccine in Venezuela by year, producer, and lot number of vaccine, 1966–68

Vaccine		Year			Total
Producer	Lot no.	1966	1967	1968	
A	37		1		1
	46		4		4
	48		2		2
	43/50[a]		1		1
	unknown	1			1
B	4		3	2	5
	5			1	1
total		1	11	3	15

[a] One case was given vaccine from two different lots.

Studies of patients' sera for different microbial antibodies, including the most common human and murine viruses, were inconclusive.[1]

In Venezuela, which has a population of approximately 9 million persons, 30–40 cases of Guillain–Barré syndrome are reported annually. Among the 20 000 persons who were vaccinated in 1967 and 1968 there were 14 cases. The authorities of the Department of Demography and Epidemiology, Ministry of Health and Social Assistance, felt that the association between the use of vaccine and the observed syndrome was statistically significant in comparison with the incidence of the syndrome among the non-vaccinated population. Because of these cases, the Pan American Zoonoses Center initiated a surveillance programme in an attempt to collect information and specimens for examination from cases that had occurred with this vaccine in any country in the past and from those that might occur in the future.

Suckling mouse brain vaccine for rabies immunoprophylaxis in man was first introduced into general use in Chile in 1963 (Table 2). In that year a total of 16 900 persons were vaccinated with 14 doses each in Chile, and an unreported number were vaccinated shortly thereafter in Uruguay. In 1969, Ecuador and Guatemala began producing SMB vaccine, making a total of at least 11 countries in which it was then employed. Each year there was a substantial increase in the use of this type of vaccine, and more than ½ million persons were vaccinated with it between 1963 and 1968.

CASES OF NEUROLOGICAL DISEASE

So far, information has been received on 40 cases of neurological disease following the administration of suckling mouse brain vaccine. Of these, 8 cases were eliminated; one was a fatal case on which no autopsy was done and the history of which could

[1] Trejos, A., Lewis, V., Fuenzalida, E., & Larghi, O. P., unpublished results.

Table 2. Number of persons (in thousands) given suckling mouse brain vaccine in Latin America by country and year, 1963–68

Country	Year [a]						Total
	1963	1964	1965	1966	1967	1968	
Chile	16.9 [b]	10.0	10.0	8.0	7.0	7.0	58.9
Uruguay	? [b]	?	?	20.0	?	?	20.0
Argentina		15.0 [b]	15.0	15.0	25.0	30.0	100.0
Peru		10.0 [b]	10.0	10.0	10.0	10.0	50.0
Brazil			20.0 [b]	25.0	35.0	35.0	115.0
Venezuela			9.6 [b]	10.0	10.0	10.0	39.6
Colombia				20.0 [b]	20.0	20.0	60.0
Cuba					? [b]	?	?
Mexico					7.0 [b]	60.0	67.0
total	16.9	35.0	64.6	108.0	114.0	172.0	510.5

[a] ? = SMB vaccine used but quantitative information not available.
[b] Year in which SMB vaccine was first used in the indicated country.

have been compatible with rabies, and the other 7 were cases for which the information submitted lacked conclusive evidence of the presence of neuropathology.

Of the 32 remaining cases considered in this report, the first occurred in Argentina in 1964, the first year in which SMB vaccine was used in that country (Table 3). After 1964, the number of cases per year increased until 1967, and since then the annual number has decreased each year. The cases occurred in several different Latin American countries, but the greatest concentration was in Venezuela.

Of the 32 cases, 7 (21.9%) presented a predominantly central nervous system disease syndrome while 22 (68.7%) involved the peripheral nervous system; in 3 cases the details were not reported. Paralysis was present in 26 cases and absent from 4, and its presence or absence was not reported in 2 cases. One of the 4 central and 6 of the 19 peripheral nervous system cases were fatal. The overall case-fatality rate was 21.9%. Autopsies were performed on 5 of the 7 fatal cases, and rabies virus could not be isolated from any of them. Demyelinization was noted at autopsy in 4 cases: the examination of the fifth case was not suitable for the detection of this type of change.

The characteristics of the neurological disease in those cases that followed the administration of other types of nervous-tissue vaccine (as reported in the literature) were compared with those of the cases that followed the use of suckling mouse brain vaccine

Table 3. Number of reported cases of neurological disease in man following antirabies prophylaxis with suckling mouse brain vaccine, January 1964–October 1969

Country	Year [a]						Total
	1964	1965	1966	1967	1968	1969	
Argentina	1 [b]	–	–	–	2	2	5
Peru	– [b]	2	–	1	–	–	3
Uruguay	– [b]	–	2	–	1	–	3
Brazil		– [b]	–	–	–	2	2
Venezuela		– [b]	1	11	3	–	15
Colombia			– [b]	–	1	1	2
Mexico				– [b]	1	–	1
Guatemala					– [b]	1	1
total	1	2	3	12	8	6	32

[a] – = no reported cases.
[b] Year in which SMB vaccine was first used in the indicated country.

Table 4. Reported cases of neurological disease in man following antirabies prophylaxis with suckling mouse brain vaccine, January 1964–October 1969: age and sex distribution

Age group (years)	Sex		Total	Percentage
	Male	Female		
0–4	1[a]	1	2	6.2
5–9	1	0	1	3.1
10–14	1	5	6	18.7
15–19	0	3	3	9.4
20–29	3[a]	2[a]	5	15.6
30–39	6[a]	2	8	25.0
40–49	3[b]	2	5	15.6
50–59	2	–	2	6.2
total	17	15	32	100.0
percentage	53.1	46.9	100.0	

[a] 1 fatal case.
[b] 3 fatal cases.

and some striking differences were revealed. Following the administration of suckling mouse brain vaccine there was a higher proportion of cases with peripheral nervous system involvement and a higher case-fatality rate. In a review of published reports (Appelbaum et al., 1953; Assis & Duchene, 1957; Gospavic et al., 1963; Pait & Pearson, 1949; Rodriguez Arias et al., 1958; Uchimura & Shiraki, 1957), data were found on 90 cases and, of these, 92.2% involved the central nervous system whereas only 21.9% of the SMB cases involved the CNS. The case-fatality rates were also different: 21.9% of the cases following the use of SMB vaccine and 4.8% of those following the use of Semple, Fermi, or Hempt vaccines. Although the percentage of cases with central involvement and the percentage of case fatalities following the use of SMB vaccine were the same, it was noted earlier that six of the deaths were patients with peripheral nervous system disease and that only one had central nervous system disease. The case-fatality rate following the administration of SMB vaccine was similar to the 20% case-fatality rate reported for Guillain–Barré syndrome from other causes.

In 15 cases, the protein and cell contents of the cerebrospinal fluid were reported. Protein contents ranged from 12 to 185 mg per 100 ml with a median of 74 mg/100 ml. The number of cells per cubic millimetre ranged from 0 to 70 with a mean of 3.

The cases occurred in all age groups from 0 to 59 years and in both sexes (Table 4): 53.1% of the cases were males and 46.9% were females. The largest number of cases was in the 30–39-year age group. Of the 7 fatalities, 1 occurred in a male 1½ years of age. The remainder occurred in persons between 20 and 49 years of age. In Latin America, 41.7% of the population is under 15 years of age, 49.5% is in the 15–59-year age group, and 8.8% is 60 years of age or over (Table 5). Information on the age and sex distribution of persons vaccinated with antirabies vaccine is not readily available. Data from three different areas (the cities of Buenos Aires, Argentina, and Lima, Peru, and the Department of Cordoba, Colombia) have been obtained and may indicate which age and sex groups are most commonly vaccinated. Buenos Aires is a somewhat uncharacteristic Latin American city in

Table 5. Percentage distribution by age and sex of the population in Latin America, of three groups of persons vaccinated against rabies, and of cases of neurological disease following vaccination with SMB vaccine

Age group (years) and sex	Population of Latin America	Groups of vaccinated persons in:			SMB "accident" group
		Argentina	Colombia	Peru	
0–14	41.7	36.7	58.1	49.3	28
15–59	49.5	55.7	41.9	47.9	72
⩾60	8.8	7.6		2.8	0
male	50	58	54.3	67.1	53.1
female	50	42	45.7	32.9	46.9

Table 6. Distribution of the reported cases of neurological disease in man following antirabies prophylaxis with suckling mouse brain vaccine by number of days to onset of symptoms January 1964–October 1969

No. of days from first dose to onset of symptoms	Cases	
	No.	%
4–6	4[a]	13.3
7–9	1	3.3
10–12	8	26.7
13–15	11	36.7
16–18	5	16.7
25	1	3.3
not reported	2	
total	32	100.0

[a] Two of these subjects had received antirabies immunoprophylaxis previously.

Table 7. Distribution of the reported cases of neurological disease in man following antirabies prophylaxis with suckling mouse brain vaccine by no. of doses received, January 1964–October 1969

No. of doses	No. of cases
5– 7	6
8–10	2
11–13	5
14	14
16	1
21	2
25	1
not reported	1
total	32

that it has a predominantly older population. The percentage of those under 15 years of age vaccinated in recent years was 36.7% in Buenos Aires, 58.1% in Cordoba, and 49.3% in Lima. Among the persons vaccinated, 35–58% were under 15 years of age. If the post-vaccinal reactions occurred uniformly in all age groups, it would be expected that 35–58% of the cases would be under 15 years of age; however, in this survey only 28% of cases were in this age group. Although actual incidence data are not available, this finding suggests that the prevalence of reactions may be considerably higher in the age groups over 15 years. In all three areas, more males than females were vaccinated against rabies, 58% of the total being male in Buenos Aires, 54.3% in Cordoba, and 67.1% in Lima. The proportion of males in the group that experienced neurological accidents was similarly higher.

The delay between injection of the first dose of vaccine and the onset of symptoms ranged from 4 to 25 days, with a median delay of 13 days (Table 6). In at least 3 cases, the patients had been given antirabies vaccine on a previous occasion: one of these had an incubation period of 5 days, one of 6 days, and one of 17 days.

Fourteen of the patients received 14 doses of vaccine and 13 received less than 14 doses (Table 7): all except one of the latter group received less than 14 doses because administration of the vaccine was suspended upon the onset of symptoms. The one exception was a patient who received 7 doses of vaccine when revaccinated after a new exposure to rabies less than 12 months after a primary course of 14 doses. This was the patient with an incubation period of 17 days.

Of the 32 patients, 7 were exposed to known rabid animals and 2 to nonrabid animals; for 23 the health status of the animal was unknown or not reported. Eighteen were bitten or scratched by the exposing animal, 3 had only contact, and for 11 the type of exposure was not reported.

Antirabies serum was administered to 2 of the patients and antitetanus serum to 4. In 14 cases no antiserum was administered and in 12 cases it was not reported whether antiserum was administered.

Nine different producers manufactured the vaccines administered to these patients. Two cases occurred in two different countries in patients treated with vaccine from the same manufacturer; only in Venezuela, however, was there more than one case associated with any one lot of vaccine.

DISCUSSION

The finding in Venezuela of such a high rate of the Guillain–Barré syndrome among vaccinated persons compared with the rate in the general population, and the temporal relationship between the administration of vaccine and the onset of symptoms in all the cases, support the hypothesis that

these cases were vaccine associated. If this hypothesis is accepted, the causative agents could be an infectious agent, a toxic product, or an allergen. Laboratory studies of the batches of vaccine concerned and of specimens from patients have been carried out and these will be reported elsewhere (Trejos et al., unpublished data). So far, none of these etiological agents has been found. Perhaps the most difficult agent to demonstrate would be an allergen. Theoretically, suckling mouse brain vaccine should contain no myelin and should produce no allergic encephalomyelitis or allergic peripheral neuritis. It is possible, however, that there are minute amounts of brain myelin present and that we are seeing the manifestations of a cross-reaction between central and peripheral nervous system tissues, or that small amounts of peripheral nerve tissue are incorporated into the vaccine and that the syndrome is an allergic neuritis provoked by peripheral nerve myelin. It is also possible that the reactions might result from the potentiation by one of the vaccine constituents of an etiological agent completely unassociated with the vaccine. It is known that the condition occurs in unvaccinated persons and perhaps the rate is higher in vaccinated persons only because of a synergistic effect. For example, contamination by microorganisms considered nonpathogenic might result in the presence of metabolic products that act as an adjuvant.

One possibility that must be considered is that modifications in the production and handling of the vaccine may be producing subtle changes in the end-product that are responsible. More than half of these 32 cases were given vaccine that was produced by methods differing, in one way or another, from the techniques originally described and tested by Fuenzalida & Palacios (1955).

Finally, it should be noted that in 1967 in Latin America, the rate of neuroparalytic accidents following the use of this vaccine was only 1 in 7 865 persons treated, in comparison with the rate of 1 in 2 844 persons treated with Semple-type vaccine.[1] It is apparently nearly 3 times as safe.

As a part of its programme, the Pan American Zoonoses Center will continue to maintain its surveillance of such complications and, in co-operation with investigators in other agencies, will try to arrive at more definite conclusions regarding the etiological factor involved in these cases.

[1] World Health Organization (1968) *World survey of rabies, IX* (unpublished document WHO/Rabies/68.167).

RÉSUMÉ

AFFECTION NEUROLOGIQUE CHEZ L'HOMME APRÈS ADMINISTRATION DE VACCIN ANTIRABIQUE PRÉPARÉ SUR CERVEAU DE SOURICEAU NON SEVRÉ

Le vaccin antirabique préparé sur cerveau de souriceau non sevré (vaccin SMB), mis au point en 1955, est le plus couramment utilisé pour l'immunisation de l'homme et des animaux en Amérique latine. Fortement immunogène, il est aisé à produire et relativement peu coûteux. En outre, comme on emploie des souriceaux âgés de 7 à 9 jours, dont le cerveau ne contient pas de myéline, on considère que ce vaccin est dépourvu de facteur encéphalitogène.

Le premier cas d'affection neurologique consécutive à l'administration prophylactique de vaccin SMB a été signalé au Venezuela en 1967. Après investigations, il est apparu que des cas similaires étaient survenus dans d'autres pays d'Amérique latine et qu'avant 1967 il s'en était produit 6 qui n'avaient fait l'objet d'aucun rapport. Au total, de 1964 à 1969 on a compté 40 accidents neurologiques suivant l'administration du vaccin. Huit d'entre eux ont été exclus de la présente étude, l'un pour diagnostic de rage douteux, les autres pour atteinte neurologique non démontrée avec certitude. Les 32 cas restants ont été observés dans huit pays d'Amérique latine, et près de la moitié au Venezuela.

Les complications dues au vaccin SMB diffèrent des accidents neuro-paralytiques observés après administration d'autres types de vaccins préparés sur tissu nerveux. Elles revêtent le plus souvent la forme d'un syndrome de Guillain-Barré. Les atteintes du système nerveux périphérique sont plus fréquentes et le taux de létalité plus élevé. Les cas surviennent dans tous les groupes d'âge et dans les deux sexes. L'incidence paraît notablement plus forte chez les sujets âgés de plus de 15 ans. Le délai entre l'injection de la première dose de vaccin et l'apparition des symptômes, qui varie de 4 à 25 jours, est en moyenne de 13 jours. Dans 3 cas au moins, les malades avaient reçu antérieurement du vaccin antirabique.

Plusieurs faits plaident en faveur de l'hypothèse selon laquelle ces accidents neurologiques sont liés à la vacci-

nation antirabique. Le facteur responsable, qui pourrait être un agent infectieux, un produit toxique ou un allergène, n'a pas été identifié. Il se peut que des modifications légères du produit final, résultant de procédés de fabrication différents de la technique originale, soient à l'origine des accidents. Il reste que la fréquence des troubles neuro-paralytiques après administration du vaccin SMB est faible et, d'après des données récentes relatives à l'Amérique latine, apparemment trois fois moins élevée qu'après emploi de vaccins de type Semple.

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