PRE-EXPOSURE IMMUNIZATION TO HUMAN RABIES WITH FUENZALIDA-PALACIOS VACCINE[1]

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The effect of Fuenzalida-Palacios antirabies vaccine on humans was studied in 33 persons. Each subject received three initial doses at intervals of one week and one maintenance dose six months later. An evaluation was made by measuring levels of neutralizing antibodies 30 and 173 days after the initial series, and 30 days after the maintenance dose.

Introduction

Recent progress in producing antirabies vaccines for human use has made possible the protection of persons consistently exposed to the risk of infection. In our region (principally in the State of Minas Gerais, where bat-transmitted urban rabies exists in endemic form) preventive use of antirabies vaccine is entirely justified to protect people exposed to transmitters.

Medical veterinarians, their assistants, and students of veterinary medicine clearly belong in this category. The risk to them was brought out by a 1968 survey of technical staff members involved in clinical work, postmortem examinations, and diagnoses at the Veterinary School of the Federal University of Minas Gerais. The survey showed that over a period of one year 27 persons had received antirabies vaccine, in series varying from 5 to 35 doses.

It was learned some years ago that a definite relationship existed between resistance to experimental rabies infection and the presence of neutralizing antibodies in the blood (*1*). This finding has been amply confirmed by research carried out on vaccinated mice, guinea pigs, dogs, and cattle (Gomez *et al.*, 1955 and Koprowski *et al.*, 1954, cited by Atanasiu) (*2*).

In 1966 the WHO Expert Comittee on Rabies affirmed the need for pre-exposure immunization of all occupational groups consistently exposed to possible rabies infection, as well as the need to subsequently determine the median neutralizing antibody levels produced (with the ultimate aim of finding the essential elements needed to introduce a safer and surer vaccination series) (*3*).

The Fuenzalida-Palacios vaccine (*4*) (with its high potency and almost complete lack of the encephalitogenic factor—according to Kabel *et al.* (*5, 6*), Thomas *et al.* (*7*), and Fuenzalida (*8*)—as well as its lack of other factors causing serious side-effects) is preferred for human vaccination, according to the opinion of the participants at the I International Rabies Seminar in the Americas (*9*). The present study was designed to determine the neutralizing antibody response to this vaccine of two types of persons: those with a history of previous antirabies vaccination and those vaccinated for the first time. The latter received a prophylactic treatment of three doses at intervals of one week and one maintenance dose six months later.

Materials and Methods

A study of neutralizing antibody titers was

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performed with sera from 33 persons between 18 and 48 years old. The subjects had been selected from a group of 303 clinically normal persons with no recent history of immunodepressive medication, who were classified as follows:

Group A consisted of 247 persons who had never received antirabies vaccinations. Out of this category we selected 30 persons at random. However, from these 30 persons we obtained only 16 complete serum samples. The immunization series consisted of three 1 ml doses (administered subcutaneously at intervals of seven days) and one maintenance dose 173 days after the initial series. Blood samples were taken before the first dose, at 30 and 173 days after the third dose, and 30 days after the maintenance dose.

Group B was composed of 45 persons with a history of antirabies vaccination, from whom we chose 10 at random. As with Group A, complete serum samples could not be obtained from all patients, so that we finally obtained only six. The immunization and serum collection schedule, as shown in Table 1, was the same as for Group A.

Group C contained 11 persons who were vaccinated with three 1 ml subcutaneous doses at intervals of 48 hours. Sera were collected 30 days after the first vaccination.

All the sera were centrifuged, bottled, inactivated at 56°C for 30 minutes, and stored at −40°C until titration was carried out. The vaccine used was type Fuenzalida-Palacios, lot No. 19, produced and kindly provided by the Oswaldo Cruz Institute, Rio de Janeiro. The titer of this vaccine, in the Habel potency test, was 647,000 LD_{50}.

TABLE 1–Previous history of vaccination against rabies of six persons from Group "B".

Code No. of Subject	No. of previous doses	Type of vaccine	Time since last vaccination
3V	10	Semple	8 months
14V	48	Semple	24 months
20V	15	Semple	7 months
23V	8	Fuenzalida-Palacios	20 days
29V	14	Semple	12 months
36V	21	Semple	24 months

Serum neutralization. The titrations were made in accordance with the technique described by Atanasiu and Johnson (*10, 11*). The challenge virus used was the strain CVS/27, provided by the Pan American Zoonoses Center, which was stored at −40°C in a 20 per cent suspension of mouse brain (in distilled water containing 2 per cent normal equine serum) inactivated at 56°C. The titer remained stable between $10^{-5.42}$ and $10^{-5.64}$ while the work was being performed, and in this way we were able to work with a variation of 30 to 50 LD_{50} between one test and another.

All sera were qualitatively tested at a 1:2 dilution. For the quantitative test, dilutions of 1:5, 1:25, 1:125, and 1:625 were used. The median value of the serum-virus titers was arrived at by the Reed and Muench method (*12*).

Results

None of the 247 persons receiving the vaccine for the first time showed any adverse reaction after vaccination. However, five local reactions were noted in the group of 45 persons previously vaccinated. These consisted of urticaria and edema in the area of the injection that diminished rapidly after antihistamines were administered.

In Group A (see Table 2) not one serum contained neutralizing antibodies on the day the first dose was administered. However, all Group A sera collected 30 days after the third dose had neutralizing antibodies, with titers varying from 1:84 to 1:220; the average titer was 1:145. Sera collected 173 days after the third dose had titers ranging from 1:13 to 1:96, with an average of 1:37. The level of antibodies had risen again 30 days after the maintenance dose; sera collected at that time (203 days after the third dose of the initial series) yielded titers from 1:72 to 1:160, with an average of 1:106.

In Group B (see Table 3) sera from the six subjects contained neutralizing antibodies before the first injection, with titers ranging

TABLE 2—Average neutralizing antibody levels of sera from 16 persons from Group "A" vaccinated for the first time, after three initial doses and one maintenance dose of Fuenzalida-Palacios vaccine.

Code No. of Subject	Average serum titers 0, 30, 173, and 203 days after the first series of vaccinations			
5	<2	198	34	94
6	<2	135	32	98
29	<2	186	38	96
30	<2	132	38	96
35	<2	>125	45	96
58	<2	180	36	98
65	<2	125	18	84
69	<2	195	32	160
74	<2	118	62	148
75	<2	84	27	159
96	<2	158	13	97
123	<2	220	96	113
129	<2	>125	32	76
133	<2	98	26	72
139	<2	84	27	109
157	<2	160	40	98

TABLE 3—Average neutralizing antibody levels of sera from six persons in Group "B", with a history of previous vaccination, who received three initial doses and one maintenance dose of Fuenzalida-Palacios vaccine.

Code No. of Subject	Average serum titers 0, 30, 173, and 203 days after the first series of inoculations			
3V	14	280	76	>125
14V	18	420	86	>125
20V	35	625	96	>125
23V	>125	>625	99	170
29V	24	320	64	>125
36V	2	345	68	364

from 1:2 to >1:125 and an average titer equal to or greater than 1:36. The titrations carried out 30 days later revealed an increased rate of neutralizing antibodies, the average titer being 1:436. One hundred and seventy-three days after the first dose, the titers dropped to an average of 1:81. However, after the maintenance dose the antibody level rose again, yielding an average titer of 1:172.

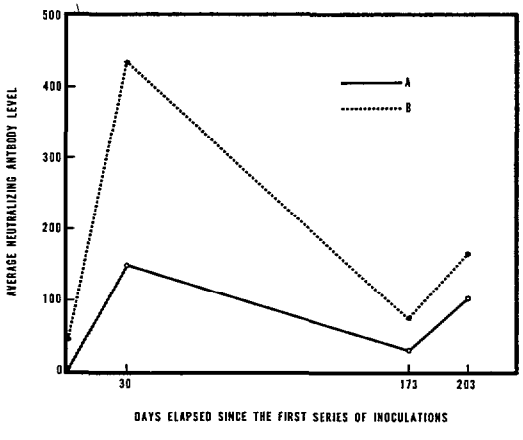
In Group C, titration of the sera was carried out 30 days after the last of the three initial vaccinations given at 48-hour intervals. Dilutions of up to 1:125 were used in these tests, the results of which are shown in Table 4.

TABLE 4—Average neutralizing antibody levels of sera from 11 persons in Group "C", vaccinated for the first time with three doses at intervals of 48 hours with the Fuenzalida-Palacios vaccine.

Code No. of Subject	Serum titer 30 days after the first dose
300	>125
301	>125
302	>125
307	>125
310	>125
311	>125
312	>125
314	>125
315	>125
317	110
320	>125

Despite the special circumstances of each Group B subject (see Tables 1 and 3), the levels of neutralizing antibodies found for Group B members differed significantly from those found for persons in Group A. However, the samples taken 173 days after the initial series showed a marked drop in both groups' antibody levels. With administration of the maintenance dose, the antibody level rose again, as may be seen in Figure 1.

FIGURE 1—Average neutralizing antibody levels in sera from Groups "A" and "B," after administration of Fuenzalida-Palacios vaccine.



Discussion and Conclusions

The results obtained for Groups A, B, and C

show satisfactory response (a neutralizing antibody level greater than 1:5) by all 33 persons given the Fuenzalida-Palacios vaccine, when tested 30 and 173 days after the initial series of inoculations. These observations are similar to those made by Fuenzalida *et al.* (*13*), Godoy (*14*), and Markus *et al.* (*15*), who also used the level of neutralizing antibodies to study the response to various series of Fuenzalida-Palacios vaccine inoculations. All of their findings showed significant neutralizing antibody levels in the sera of vaccinated persons.

However, this consistently satisfactory antibody response has not been substantiated for other types of antirabies vaccines. Fuenzalida *et al.* (*13*) only obtained a 39 per cent positive response from individuals who received a vaccine produced in the brain of an adult rabbit. Atanasiu *et al.* (*16*), working with Flury HEP, duck embryo, and Semple vaccines, obtained an approximately 50 per cent positive response 30 days after inoculation. Greenberg and Childress (*17*) obtained a 97 per cent positive response after 21 and 60 days from individuals who received 14 doses of Semple vaccine. The average level of neutralizing antibody titers was 1:75. With duck embryo vaccine the response was 93 per cent positive and yielded an average titer of 1:40. Shipley and Jubett (*18*) obtained a 77.8 per cent positive response in 644 persons who received two to three doses of duck embryo vaccine at one-week intervals. Titers of 1:5 or more were considered positive.

Sera from Group B, which was comprised of previously vaccinated persons (see Tables 1 and 3), showed increased levels of neutralizing antibodies at 30, 173, and 203 days after the first series of inoculations. According to Davis *et al.* (*19*) this secondary response can be distinguished from a primary one by the following characteristics: a shorter negative phase, a lesser quantity of immunogen needed to induce antibody formation, and more persistent antibody formation.

Comparison of the titers of sera from those with a history of previous vaccination and those vaccinated for the first time (see Tables 2 and 3) shows that those with greater previous exposure to the antigen (see Table 3) responded better than those with less exposure.

Pereira *et al.* (*20*) studied secondary response in 87 instances of antirabies revaccination and concluded that the time elapsed since the last vaccination did not influence the results, but that the number of doses administered was significant. Cohen *et al.* (*21*) studied the response to a "booster" administered after one year to 20 individuals, who received four doses of duck embryo vaccine at one-week intervals, and found a significant rise in the levels of neutralizing antibodies 1, 2, 5, and 12 days after vaccination.

SUMMARY

In order to determine resulting levels of neutralizing antibodies against rabies, the degree of antibody persistence after six months, and the effects of a booster shot, experiments were conducted with Fuenzalida-Palacios vaccine. Sera from 33 persons were studied. Those who had previously been vaccinated against rabies received three vaccinations at one-week intervals and a booster six months later.

The results obtained from these studies were as follows: the administration of the three initial doses at one-week intervals produced a positive response in all persons studied. The individuals previously vaccinated against rabies showed a booster-type response to their initial series of vaccinations. One hundred and seventy-three days after administration of the three-dose series, however, members of both groups showed a marked reduction of neutralizing antibody levels, though these were still at titers considered satisfactory.

On the basis of these results, it is concluded that the booster is justified after six months, since it produces a new increase in the rate of neutralizing antibodies. Also, three-dose prophylactic immunization, administered at either 48-hour or one-week intervals, seems to produce satisfactory results.

The two individuals who had received Semple vaccine showed a response similar to that obtained with the booster shot.

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