EFFECT OF SITE OF INJECTION OF ANTIRABIC VACCINE ON IMMUNITY FORMATION

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Tests of intracerebral, intraperitoneal, intramuscular, and subcutaneous immunization of mice with cultural antirabic vaccine and of immunity with street rabies virus showed that the highest protection is obtained by injection of antigen into the brain. After injection of the vaccine into the subcutaneous cellular tissue of the lip, the sole of the hind limb, the thigh, the dorsum, abdomen, and neck,the intensity of immunity was highest in those mice which were immunized in the lip and sole. The effectiveness of antirabic vaccination thus depends on the high concentration of nerve endings at the site of injection of the antigen.

Despite much research the mechanism of active immunity against rabies has not yet been adequately studied. The effectiveness of antirabic vaccination is known to depend largely on the mode and site of injection of the vaccine [1, 3-6, 9, 10, 13, 16]. However, these investigations were restricted to the study of the effectiveness of two or, less frequently, three methods of immunization.

Intracerebral immunization is of theoretical interest. Before 1954 attempts to immunize animals by injecting virus or vaccine directly into the brain had given negative or unconvincing results [1, 5, 9, 14, 15]. Since 1954 a number of workers have obtained conclusive evidence that intracerebral immunization of animals can be achieved with fixed rabies virus of the strain Flury HEp [7, 9, 11, 12]. However, this virus, which is nonpathogenic for some adult animals, successfully immunized animals if the antigen was injected into the brain, but produced little or no immunization of laboratory animals by other methods of vaccination. It was thus impossible to assess the effectiveness of intracerebral immunization and to compare it with other methods of vaccination.

The writers have studied the effectiveness of antirabic vaccination in experiments on albino mice in relation to the site of injection of the antigen. The study of this problem is interesting both from the standpoint of explaining the genesis of antirabic activity and also for elucidation of the optimal conditions for the use of antirabic vaccine. Two batches of cultural antirabic vaccine from the Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, were used to immunize the animals. Batch no. 102 was completely inactivated, with an index of immunogenicity (Habel) of 3981; batch no. 13 had a residual infectious titer of 3.4 log LD_{50} 0.03 ml, ED_{50} 0.4 μl. Albino mice weighing 13-14 g were immunized by injection of vaccine into different tissues in different parts of the body. The course consisted of two injections, each of 0.25 ml vaccine, in dilutions of 1:10 or 1:20, at an interval of seven days. The total dose of vaccine was thus the same as for extraneural vaccination. The resistance of the mice was tested 13-15 days after the beginning of immunization by infection of the animals with a known lethal dose of street rabies virus, strain Mochalin into the subcutaneous cellular tissue of the lip (in some experiments the infecting dose was 8 LD_{50}, in others 16-32 LD_{50}). The experimental results were assessed with respect to the survival rate of the mice in percent and the serum antibody titer. The neutralization test and calculation of the antibody titer were carried out as described previously [8]. The difference between the survival rates of the two groups of animals were taken to be statistically significant when $P < 0.05$ [2].

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TABLE 1. Determination of Protective Action of Cultural Antirabic Vaccine in Relation to Site of Application

Experiment no.	Site of injection of vaccine	Total number of mice	Rate of Survival (in percent)	Titer of antibodies
1	Into the brain	46	100	Not tested
	Intraperitoneally	45	78	
	Into the thigh muscles	50	50	
	Into dorsal subcutaneous cellular tissue	50	0	
	Control	50	0	
2	Into the brain	21	100	1:250
	Intraperitoneally	18	44,5	1:17
	Control	20	0	0
3	Into the brain	28	100	1:1 400
	Intraperitoneally	30	90	1:96
	Control	30	0	—
4	Subcutaneously into the lip	50	100	Not tested
	Subcutaneously into sole of hind limb	50	98	
	Into the thigh muscles	50	56	
	Subcutaneously into thigh	50	22	
	" " abdomen	49	10	
	" " dorsum	48	0	
	" " neck	49	0	
	Control	50	0	

Note. 1) Vaccine of batch no. 102 was used in experiments 1-3 and batch no. 13 in experiment 4. 2) In experiment 3 the mice were immunized four times at intervals of seven days. The resistance and antibody titer were investigated on the 30th day after the beginning of the experiment. 3) In experiments 1-3 the infecting dose of street virus was 16 LD_{50}, and in experiment 4 it was 32 LD_{50}. 4) In experiment 2 the sera were tested against 74 LD_{50} of fixed rabies virus strain CNS, and in experiment 3 against 627 LD_{50}.

EXPERIMENTAL RESULTS

In a preliminary experiment the effectiveness of immunization of the mice into the brain, the peritoneal cavity, the thigh muscle, the skin of the abdominal wall, the subcutaneous cellular tissue in the dorsal region, and into the caudal vein was compared. While all the animals in the control group developed the disease, the highest protection was found after intracerebral vaccination (10 of the 11 mice survived) and the lowest by subcutaneous immunization in the dorsal region (only four of the 15 mice survived). After intramuscular, intraperitoneal, intravenous, and intradermal vaccination the level of immunity was lower than after intracerebral vaccination.

These results were confirmed in the next experiment (Table 1, experiment 1). A sharp difference was observed between the effectiveness of intracerebral and subcutaneous immunization. Whereas all the mice survived after intracerebral immunization, subcutaneous immunization gave no protection whatever. It was possible to detect these fluctuations because the immunity was tested under more rigorous conditions (dose of street virus 16 LD_{50} in 0.1 ml). After intraperitoneal and intramuscular immunization, 78% and 50% of the mice respectively were protected. The differences are statistically significant ($P < 0.05$). Titration of the sera of the mice immunized intracerebrally and intraperitoneally (Table 1, experiments 2 and 3) showed direct correlation between the resistance of the animals to street virus and the serum antibody titer: complete protection of the mice vaccinated intracerebrally and a high antibody titer (1:250) but comparatively low resistance in the mice vaccinated intraperitoneally (survival rate 44.4%) and a low serum antibody titer (1:17). The difference is statistically significant ($P < 0.05$). Finally, the results of experiment 3 show that after quadruple immunization high protection was obtained with both methods of immunization (the difference is not statistically significant). Meanwhile the results of titration of the sera showed that the blood antibody level of the intracerebrally immunized mice was 14.6 times higher (1:1400) than in the mice immunized intraperitoneally (1:96). No corresponding data could be found in the accessible literature. Parallel with the sera, the brain of the immunized mice also was investigated to detect tissue virus-neutralizing anti-

bodies. At all times of investigation (7, 14 and 30 days after the beginning of immunization) negative results were obtained.

Injection of vaccine into the brain thus led to the formation of antirabic immunity of the highest intensity. It must be emphasized thet the high resistance after intracerebral immunization was not due to tissue virus-neutralizing antibodies. Evidently serum virus-neutralizing antibodies play an essential role in antiviral antirabic immunity, as is shown by the correlation found between the antibody titer and the resistance of the mice to street virus.

The results of these experiments indicate the important role of the central nervous system in the genesis of antirabic immunity. To examine the role of the peripheral nervous system, animals were immunized by injection into the thigh muscles and into the subcutaneous cellular tissue in different parts of the body. The results of one of the three similar experiments are given in Table 1 (experiment 4). The intensity of immunity was found to be closely related to the density of nerve endings at the site of injection of the vaccine. In all the experiments high protection was found after injection of the vaccine into the subcutaneous cellular tissue of the lip and into the sole of the hind limb. Distance to the central nervous system was of no importance. This was shown by the absence of protection in mice vaccinated by injection into the subcutaneous cellular tissue of the neck.

It is well known that in order to produce rabies in laboratory animals the most important factor is that the virus must be injected into the brain and into tissues richly supplied with nerve endings. The same characteristic features were found in these experiments with respect to the formation of active antirabic immunity. This fact suggest that the ways of spread and the ultimate localization of the virus are probably identical during development of the disease and in immunization.

It can be concluded from the results of these experiments that the intramuscular method of immunization is advisable if completely inactivated antirabic vaccine is used.

LITERATURE CITED

1. M. I. Akker, Transactions of Scientific Research Institutes of Kuibyshev Province and Kuibyshev Medical Institute [in Russian], Vol. 1, Kuibyshev (1935), p. 118.
2. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Research [in Russian], Leningrad (1962).
3. D. E. Zibitsker and N. A. Kovalev, in: Rabies and Its Prevention [in Russian], Minsk (1968), p. 50.
4. D. E. Zibitsker and V. I. Korotkevich, in: Collected Scientific Transactions of the Belorussian Institute of Epidemiology, Microbiology, and Hygiene [in Russian], Minsk (1955), p. 168.
5. K. P. Muratova and N. P. Dedova, Zh. Mikrobiol., No. 1-2, 137 (1942).
6. E. N. V. Nagorenko, in: Rabies [in Russian], Moscow (1958), p. 172.
7. M. A. Selimov and L. I. Kalinina, in: Rabies and Its Control [in Russian], Moscow (1967), p. 231.
8. M. A. Selimov, E. V. Klyueva, and E. V. Semenova, Modern Methods of Laboratory Diagnosis of Rabies [in Russian], Moscow (1964), p. 43.
9. M. M. Tsekhnovitser and L. Ya. Gol'denberg, Zh. Mikrobiol., 7, No. 2, 141 (1930).
10. K. Habel, Public Health Rep. (Washington), 60, 545 (1945).
11. Q. Huygellen, J. Hyg. (London), 58, 187 (1960).
12. H. Koprowski and J. Black, J. Immunol., 72, 503 (1954).
13. H. Koprowski, J. Black, and W. Johnson, J. Am. Vet. Med. Ass., 127, 363 (1955).
14. A. Marial and S. Muttermilch, C. R. Soc. Biol., 98, 15 (1928).
15. P. Remlinger and J. Bailly, Ann. Inst. Pasteur, 42, 729 (1928).
16. L. Webster, Am. J. Public Health, 26, 1207 (1936).