POSTVACCINAL REACTION IN *Papio hamadryas* TO INJECTION OF ATTENUATED VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS (STRAIN 15)

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Among viruses, during working with which in the laboratory infection of the staff is most likely to happen, due to inhalation of a virus-containing aerosol, one of the leading places is occupied by Venezuelan equine encephalomyelitis (VEE) virus [2, 11, 13]. Starting with the 1930s there has been a steady rise in the number of recorded cases of infection taking place within the laboratory, and the fastest from 1963 through 1967 (affecting more than 70 people in this last period) [12].

The use of an attenuated strain TC-82 (USA) and its subsequent derivatives as living vaccine has succeeded in arresting this increase of diseases acquired in the laboratory. However, the results of tests of living vaccines based on strains of the TC series show that, besides high immunogenicity and protective activity [6, 7], they are also capable of giving rise to clinically manifest postvaccinal side reactions in more than 20-30% of subjects vaccinated [5]. Vaccines based on inactivated virus [7] and on living recombinant virus [8] have been tested in recent years but do not confer reliable protection on animals against infection by a virulent strain of VEE virus through the respiratory tract. Ultimately, considering the high level of risk during work with this agent, which can cause the disease as a result of inhalation of a virus-containing aerosol [2, 6], reactogenic strains of the TC series as before continue to be used to protect workers in research establishments.

Accordingly, a search for and evaluation of other attenuated strains are being conducted in the Research Institute of Microbiology, Ministry of Defense of the USSR. In a preliminary series of experiments we demonstrated the high protective value of one of these, namely attenuated strain 15, in the case of aerogenic infection of rodents. The next stage of the experimental evaluation consisted of experiments with vaccination of primates, to determine the frequency of appearance of individual symptoms of the postvaccinal reaction and their severity.

The baboon *Papio hamadryas,* which had previously been used at the above-mentioned Institute by Yu. V. Chicherin and co-workers [4] to evaluate vaccine preparations against the plague bacillus [3], was chosen as the model animal.

EXPERIMENTAL METHOD

A culture of strain 15 of VEE virus was obtained from the Museum of Vaccine Strains, Research Institute of Microbiology, Ministry of Defense of the USSR, in the form of a sublimation-dried homogenate of chick embryos, rehydrated before use with sterile physiological saline. Strain Trinidad (IA) was obtained from the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, and working cultures of the virus were prepared by infection and subsequent homogenization of chick embryos.

Immunization was carried out by subcutaneous injection of the attenuated strain, and in all cases the doses were calculated by the value of ImD_{50} for guinea pigs (the dose giving protection to 50% of guinea pigs against infection by the virulent Trinidad strain).

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TABLE 1. Frequency of Appearance of Postvaccinal Reaction in *P. hamadryas* after Immunization with Strain 15, and also of Symptoms of Disease after Aerogenic Infection with Strain Trinidad ($P \pm \sigma_p$)

Symptom	Number of animals with symptoms of the disease, their relative number, and mean error, %		
	injection of strain 15 in dose of 10^3 ImD_{50} (six animals)	injection of strain 15 in dose of 10^6 ImD_{50} (four animals)	infection with strain Trinidad in dose of 10-30 AID_{50} (six animals)
Viremia	2 (33±21)	2 (50±29)	6 (100—17)
Fever	2 (33±21)	2 (50±29)	6 (100—17)
Disturbance of CNS activity	0 (0±17)	0 (0±25)	2 (33±21)
Meningeal symptoms	0 (0±17)	0 (0±25)	1 (17±17)
Depression (apathy, refusal to eat, inhibition)	0 (0±17)	0 (0±25)	6 (100—17)

Legend. All animals had neutralizing antibodies in their blood serum in titers of 1:125 or higher 1 month after vaccination or infection.

TABLE 2. Duration and Severity of Symptoms Characterizing Postvaccinal Resction after Injection of Strain 15, and Disease Developing in Baboons after Infection with Strain Trinidad ($\bar{X} \pm \sigma_{\bar{x}}$)

Parameter	Injection of strain 15 in dose of 10^3 ImD_{50}	Injection of strain 15 in dose of 10^6 ImD_{50}	Infection with strain Trinidad in dose of 10-30 AID_{50}
Duration of viremia, days	1,5±0,4	1,5±0,4	3,0±0,5
Duration of fever, days	1,0±0,0	1,0±0,0	3,0±0,5
Maximal rise of temperature ($T°C_{max} - T°C_{norm}$)	0,2±0,0	0,4±0,0	1,2±0,1
Duration of period in which neurological disorders were observed (tremor, seizures, pareses), days	—	—	3,5±0,4
Duration of depression, days	—	—	3,6±0,7
Duration of illness (from first symptoms until clinical recovery), days	1,0±0,0	1,0±0,0	5,7±0,7

Legend. $\sigma_{\bar{x}}$) Error of mean; —) no symptoms present.

In experiments with aerogenic infection of monkeys, the dose used was expressed in AID_{50} units (quantity of virus causing the disease in 50% of animals) The percentage of animals reacting to vaccination or infection, and also the mean error of the percentage ($p \pm \sigma_p$) were determined by the use of V. S. Genes' tables [1].

Baboons weighing 5-7 kg were kept for not less than 30 days in the animal house in communal cages, and immediately before the experiment they were kept for 5-7 days in individual cages, for the purpose of inspection. The animals showing signs of the disease or trauma were discarded.

EXPERIMENTAL RESULTS

The reaction of the baboons to administration of strain 15 in doses of 10^3 ImD_{50} (six animals), 10^6 ImD_{50} (four animals), and also to aerogenic infection with strain Trinidad in doses of 10-30 AID_{50} (six animals) was studied. The results, given in Tables 1 and 2, show that attenuated strain 15 in a dose of 10^3 ImD_{50} did not cause any serious clinical manifestations of the disease in the baboons. About 30% of the animals developed transient viremia and a mild febrile reaction. Symptoms indicative of a lesion of the CNS were not present. All the animals developed an immune response, detectable by the appearance of virus-neutralizing antibodies in the blood sera in titers of 1:125 or higher.

A 1000-fold increase in the immunizing dose (up to 10^6 ImD_{50}) led to an increase in the relative number of animals responding by viremia and fever, but this was not accompanied by any appreciable change in these parameters.

Unlike the animals vaccinated with strain 15, baboons infected with an aerosol of strain Trinidad (doses 10-30 AID_{50}) developed a manifest disease, characterized by more prolonged viremia and fever, apathy, and inhibition. Two of the six animals were observed to have symptoms indicative of a lesion of the CNS (tremor of the limbs, seizures affecting individual muscle groups). In one of them paresis of the lower limbs was observed, in another — rigidity of the neck muscles accompanied by a

characteristic posture, namely with the head thrust forward. The symptoms observed were transient in character and disappeared by the 10th day after the appearance of clinical features of the disease.

The results are evidence that strain 15 does not cause any marked illness in *P. hamadryas* (by contrast with the natural Trinidad strain) and does not give rise to clinically detectable complications affecting the CNS, while producing seroconversion in virtually all the vaccinated animals Nevertheless, despite the short duration and mildness of the postvaccinal reaction, fever was observed in about 30% of the baboons vaccinated with a dose of 10^3 ImD_{50}.

In connection with the above remarks it is interesting to compare these results with those obtained previously by American workers studying personnel infected as a result of airborne infection with a virulent strain, and alsd in a study of vaccines of VEE virus based on an attenuated strain of the TC series on volunteers. Comparison of two groups of people (vaccinated [5] and having had the disease [9, 10]) showed that during development of the postvaccinal reaction (in approximately 30% of volunteers) the duration of the symptoms averaged under 2 days, whereas in the group developing the disease, accompanied by marked fever, it was about 6 days; in some cases, moreover, dizziness and loss of balance, inhibition, loss of appetite, severe headaches, rigidity of the neck muscles, delirium, and also residual manifestations in the form of tremor of the hands, asthenia, and loss of olfaction, were observed. No disturbances affecting the CNS were found in the vaccinated individuals, and their febrile reaction was mild (mean duration about 1 day).

Analysis of the clinical manifestations observed in the baboons after vaccination with attenuated strain 15 or after aerogenic infection with strain Trinidad shows that the trend of the differences between these groups as regards the frequency of appearance of the symptoms and their duration is on the whole identical with that observed in man This sufficiently close coincidence suggests the similarity of the course of the vaccinal and infectious processes in these animals and in man, and it means that *P. hamadryas* can be regarded as a model object with which to study living vaccines of VEE virus.

The investigation thus showed that strain 15, while not giving rise to any characteristic manifestations of the disease, when injected subcutaneously into *P. hamadryas,* such as accompany infection with the virulent Trinidad strain, induces an immune response detectable by positive seroconversion in all the animals. This fact is evidence of attenuation of the strain while preserving its high immunogenicity. However, a postvaccinal reaction, in the form of moderate and transient fever, is observed in 30% of monkeys vaccinated with a dose of 10^3 ImD_{50}.

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