INCREASED SENSITIVITY OF ALBINO MICE TO NATURAL SMALLPOX VIRUS AFTER WHOLE-BODY γ-RAY IRRADIATION

S. S. Marennikova and R. A. Shafikova

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Preliminary whole-body irradiation of albino mice with γ rays in a dose of 400 R increased their sensitivity to intracerebral injection of natural smallpox virus. The infection subsequently developing was similar in its clinical picture to that in unirradiated mice of a naturally susceptible age (12-15 days). Following injection of virus into irradiated animals intranasally and intravenously, no increase in or development of susceptibility was observed.

An increase in or the development of sensitivity to vaccinia virus in naturally resistant animals after preliminary γ-ray irradiation has been described in the literature [1-4, 6].

With these facts in mind, and remembering that only very young mice which are not always suitable for experimental work (for example, for the testing of immunity), are susceptible to natural smallpox virus under normal conditions, in the present investigation whole-body γ-ray irradiation was used in an attempt to increase the susceptibility of albino mice to natural smallpox virus.

EXPERIMENTAL METHOD

Experiments were carried out with natural smallpox virus (strain M-T-60) after two preliminary passages through chick embryos. The material for infection consisted of a suspension of the choriollantoic membranes of infected chick embryos. Noninbred albino mice aged 28-45 days were used in the experiments. The mice were irradiated in a biological γ-ray apparatus (GUBÉ-800, dose rate 126 R/min) in a dose of 400 R, and 24 h later they were infected in various ways: intranasally, intracerebrally, and intravenously, after which they remained under observation for 10-14 days. Irradiated, uninfected mice and irradiated mice receiving an injection of a suspension of normal choriollantoic membrane served as controls. The specificity of the infection in the mice was confirmed by isolation of the virus from their brain, lungs, and kidneys on the chorioallantoic membrane of chick embryos. The dynamics of accumulation of virus in the blood and organs of the irradiated mice was studied by the usual method [5].

EXPERIMETNAL RESULTS

The albino mice used in the whole-body γ-ray irradiation experiments were aged 28-45 days, i.e., much older than mice sensitive under ordinary conditions to infection by natural smallpox virus (12-15 days). The mice aged 28-31 days were subdivided into 3 groups 24 h after irradiation and were infected as follows: group 1 intracerebrally, group 2 intranasally, and group 3 intravenously. Since mice are much less sensitive to virus when administered intranasally and intravenously, mice over 30 days old were infected only intracerebrally. The results showed that irradiation of mice aged 28-31 days does not lead to the development of sensitivity of the animals to intranasal and intravenous administration even of concentrated suspensions of natural smallpox virus. At the same time, following intracerebral inoculation of the virus into irradiated mice of the same age, a disease picture on the whole similar to that when this same

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Laboratory of Smallpox Prevention, Institute of Virus Preparations, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 8, pp. 102-105, August, 1970. Original article submitted September 22, 1969.

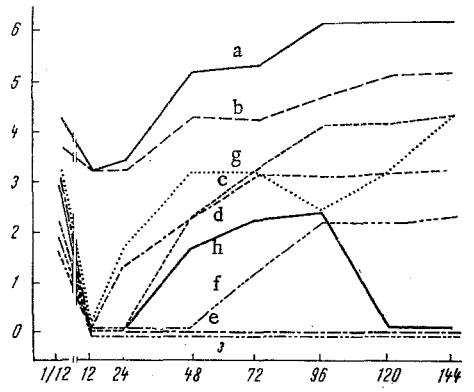

Fig. 1. Accumulation of virus in blood and organs of irradiated mice: a) brain; b) spinal cord; c) lungs; d) heart; e) liver; f) kidneys; g) blood; h) spleen; i) testicles. Abscissa, time (in hours); ordinate, dose of virus (in $\log_2$ VFU).

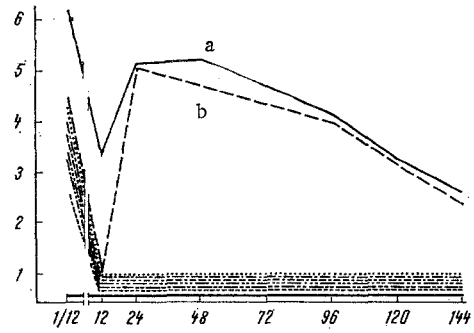

Fig. 2. Accumulation of virus in blood and organs of unirradiated mice. Legend as in Fig. 1.

method was used on unirradiated mice aged 12-15 days was observed. The only difference was that in the first case, the animals additionally developed conjunctivitis and they died earlier after infection. No signs of disease were found in the control animals. Virological investigation of the irradiated animals which died from specific infection revealed the presence of virus in the brain, lungs, and kidneys, despite the very slight nature of the macroscopically visible pathological changes: anemia and a sharp decrease in size of the spleen, together with foci of pneumonia at the root of the lungs. Similar changes in the spleen were also found in the control animals (irradiated mice receiving a suspension of normal chorioallantoic membrane), although these animals did not die.

The development of sensitivity of the irradiated mice to intracerebral injection of natural smallpox virus was possible only up to a certain limit. For instance, whereas log LD_{50} for mice aged 28-31 days is 2.76, for mice aged 34-37 days it was reduced to 1.5. Mice aged 40-45 days were clinically completely immune to injection of the virus.

In the next series of experiments the dynamics of development of the disease and of accumulation of virus in the blood and organs of mice aged 28-31 days was studied after intracerebral infection [dose of virus 5×10^4 vesicle-forming units (VFU) (0.05 ml)]. Unirradiated mice infected with the same dose of virus acted as the control. The results obtained are shown in Figs. 1 and 2.

It will be clear from Figs. 1 and 2 that 5 min after infection virus was found in the blood and in all the organs except the testicles; after 12 h it was found only in the spinal cord and brain. Starting from 24 h after infection, a gradual increase in the quantity of virus was found in the blood, spinal cord, brain, lungs, kidneys, and heart of the irradiated mice, to reach a maximum by 120-144 h. The highest concentration of virus in the testicles was found after 96 h, after which the quantity decreased. Neither in the liver nor in the spleen was any accumulation of virus observed. When these results are compared with those of investigation of unirradiated mice of the same age, it was found that in the latter group, despite an identical content of virus 5 min after infection, its pattern of distribution differed significantly: only a very slight accumulation of virus occurred after 24-48 h in the spinal cord and brain, and a complete absence of its accumulation in the other organs and blood.

According to data in the literature [5, 7], the susceptibility of albino mice to natural smallpox virus is determined by age: after intracerebral infection it is limited to 12-15 days, and after intranasal infection, to 1-2 days. The results of the present experiment show that, by using whole-body irradiation with Co^{60} γ rays, the age threshold of susceptibility to natural smallpox virus by the intracerebral method of infection can be raised to 31-37 days (by 2-2.5 times). The clinical course of the infection and accumulation of the virus in organs of the irradiated animals were virtually indistinguishable from those observed in mice aged 12-15 days without preliminary irradiation: in both cases a primary and secondary viremia was observed, with accumulation of virus in the brain, spinal cord, lungs, kidneys, and heart; no virus was found in the liver and spleen.

Development of smallpox infection in mice aged 28-31 days did not take place after intranasal and intravenous administration of the virus, despite the use of the same method of preliminary whole-body γ-ray irradiation. Since in these experiments mice more than 15 times older than the age of natural suscepti-

bility were used, it follows that to detect the phenomenon of increased susceptibility to intranasal infection mice aged 4-5 days should be used – i.e., mice 2-3 times older than the age of natural susceptibility.

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