

Immunizing potential of vaccinia virus inactivated by ultraviolet rays

by Y. Nagano and Y. Kojima.

[1] In a precedent paper (1*), we have indicated that the vaccinia infection experiments of the rabbit skin can be inhibited by the intradermal injection of the equivalent virus inactivated by ultraviolet irradiation. [2] We have tried next, on one hand, to see if the complete virus is the sole inhibitory factor contained in the virulent material. [3] On the other hand, regarding to know if it acts in classical immunity or phenomenon of so-called interference, we have searched to compare the antigenic strength in vivo and the inhibitory effect of the vaccine materials submitted to different treatments.

1. Inhibitory effect of the inactivated dermo-vaccine. — [4] As we have indicated precedently, the dermo-vaccine prepared 3rd days after the inoculation possessed an inhibitory effect less than the testis-vaccine containing the same number of infecting units.

[5] But, when we prepared the dermo-vaccine at 5th day after the inoculation, the inhibiting strength was found more than the material prepared at 3rd day, while the virulence was equal to that of the material at 3rd day. [6] These results suggested the presence, in infected tissues, of one or several factors, non-virulent but being able to inhibit the infection.

2. Influence of centrifugation on the inhibitory effect. — [7] With the intention of determining if the complete virus is the sole inhibiting factor, we centrifuged the dermo-vaccine at 6,000 or 35,000 rounds-minute for 30 to 60 minutes.

Sample	Virus titer	Irradiation time in minute			
		0	2	6	10
Initial dermo-vaccine	10 ⁶			10	1 - 10
Supernatant	10 ⁴		10	1 - 10	1
Precipitation (virus)	10 ⁶		10	—	—

Inhibitory effect (maximum effective dilution) of dermo-vaccine
centrifuged for 30 min at 6,000 rounds-minute.

[8] The depot, newly suspended in a solution mopped by phosphates, contained 10^6 to 10^7 infecting units by 0.2 ml. [9] When the thus partially purified virus was irradiated for 2 minutes, minimum time for the complete inactivation, it kept an inhibiting strength also the same as the non-centrifuged suspension. [10] Nevertheless, an irradiation for 6 minutes eliminated totally the inhibiting strength.

[11] The supernatant contained only 10^4 to 10^5 infecting units by 0.2 ml. Despite so weak virulence, the supernatant showed, after irradiation, so effective in the inhibition test as the original suspension. [12] Furthermore, even to 10 minutes, the excess irradiation of the supernatant did not eliminate its inhibiting effect.

[13] We asked ourselves then if the original virulent suspension contains, besides infecting virus, factors non-infecting but providing with inhibiting property, or if there are constituents that protect the virus against the harmful action of the excess irradiation. [14] Regarding to clarify this point, we have first irradiated in excess the virulent suspension, then centrifuged. [15] The supernatant was found, in the inhibition test, as effective as the original suspension, while the depot was much less effective. [16] It was resulted that the virus suspension contains factors non-infecting but possessing the inhibiting strength, and that these factors are more resistant to the irradiation in excess than the complete virus particles.

3. Production of neutralizing antibodies by the mouse previously received with the vaccine material. — [17] The inhibitory effect described above would be the immunizing property of the virulent suspension? [18] Which animal would be chosen for determining precisely the immunizing strength of vaccine materiel? [19] By preliminary assays, we have found that, in contrast to the rabbit and to the guinea pig, the mouse is not immunized spontaneously very rarely.

[20] Groups of six mice received a sole dose of dilutions of vaccine materiel by the intra-peritoneal way. [21] To the end of 3 to 5 weeks, the animals were healthy. [22] The serums of every group animals were collected and diluted to the fifth. [23] The reaction of neutralization was performed according to the technique previously described. (2*). [24] We thus confirmed the following facts: a) [25] The dermo-vaccine or the testis-vaccine, partially purifies or not, provokes with the mouse the antibody production so as the inoculum contains at least 10^6 units of infecting viruses. [26] The infection of 10^5 units infectants provokes very difficultly appearance of antibodies. [27] With 10^4 units, one cannot induce the antibody production. b) [28] The ultraviolet irradiation completely can inactivate the dermo-vaccine without weakening of its immunizing strength.

4. Immunizing potential of the supernatant after centrifugation of the dermo-vaccine.

— [29] The supernatant after centrifugation of the dermo-vaccine shows, as we indicated in the experiment n^o. 2, so effective in the inhibition test as the initial suspension despite it contains very small viruses. [30] We tried to immunize the mouse with the supernatant containing only 10⁴ units of infecting viruses. [31] The result was positive. [32] This can be the proof that there are in the supernatant constituents non-infectious but providing the immunizing strength. [33] This observation confirms some of the works of Nakamura and his colleagues (3*).

Discussion. — [34] For the moment, we cannot determine if the inhibition of the skin infection by the irradiated virus is an immunological phenomenon or so-called interference. [35] Nevertheless, if one examines the relations between the inhibiting strength and the immunizing potential of vaccine materials obtained by different treatments from different tissues, one observes, when the inhibiting strength is big, the immunizing strength is also big, when the first one is small or null, the second is likewise small or null in parallel.

[36] From the practical point of view, it is important that the inactivated virus possesses a high immunogenic strength, since it suggests the possibility of preparing an inactivated vaccine against smallpox.

Resume. — 1) [37] The mouse suits for determining the antigenic strength of the vaccine materiel. [38] With this animal, the minimum immunizing dose of virus inactivated by ultraviolet rays was found equal to that of the active virus.

2) [39] The tissue infected by the vaccinia virus contains constituents not infecting but providing with properties to inhibit the dermal infection with the rabbit and to provoke the production of the neutralizing antibodies with the mouse. [40] The factors are not precipitated by a centrifugation for one hour at 35,000 rpm.

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(1*) Y. Nagano, Y. Kojima et Y. Sawai, *C. R. Soc. Biol.*, 1954, **148**, p.750.

(2*) Y. Nagano, *Japan. J. Exp. Med.*, 1949, **20**, p.145.

(3*) Y. Nakamura, *Acta Medica Hokkaido*, 1937, **15**, p. 2789.

-----[translated by Yoshihiko WATANABE on 23/3/2004]-----