Y. Nagano et Y. Kojima: Inhibition de l'infection vaccinale par un facteur liquide dans le tissu infect par le virus homologue. *C. R. Seances Soc. Biol. Fil.* **152**: 1627-1629 (1958).

Inhibition of the infection of vaccinia virus by a liquid factor in the tissues infected by the homologous virus,

by Y. Nagano and Y. Kojima.

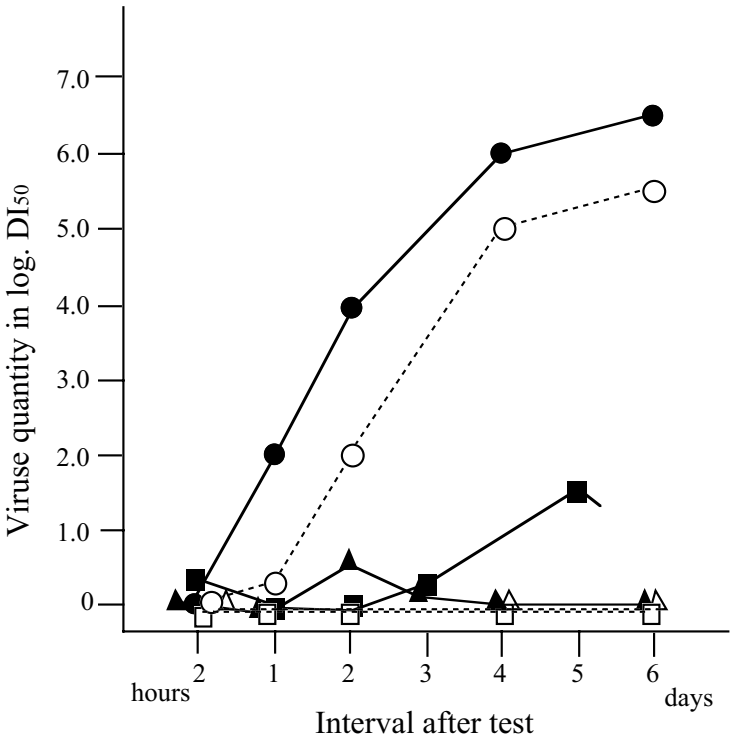
[1] In the precedent report (1), we have described the interference of the inactive vaccinia virus with the equivalent active virus. [2] In the course of these researches, we have found that the infection can be inhibited not only by virus particles, but also by the liquid portion of the brayed infected tissue. [3] In the present report, we will express some experimental results concerning the liquid factor.

Preparation of the supernatant of the brayed infected tissues. — [4] The skin of the rabbit was inoculated with the caccinia virus by scarification. [5] At the fifth day, the virulent materiel was prepared according to Parker and Fasten (2); the inoculated skin was scraped with a spatula. [6] The turbid suspension obtained was centrifuged for 15 minutes at 3,000 rpm. [7] The supernatant was preserved at - 20 °C. [8] Before using the supernatant, it was centrifuged for 30 minutes at 12,000 rpm. [9] To totally remove the infectivity of the supernatant, centrifugation was repeated three times.

Inhibition by the supernatant of the vaccinia infection. — [10] The supernatant was injected to the rabbit by the intradermal way. [11] As a control, an extract of the non-infected tissues was injected. [12] The injection places were marked with dye. [13] At the end of 24 hours, dilutions of the virulent materiel were injected to the marked places. [14] The animals were observed for 14 days. [15] At the control places, the DI_{50} showed values from $10^{-7.5}$ to $10^{-8.5}$. [16] The preparative injection of the supernatant dropped the DI_{50} to $10^{-4.5}$ or to $10^{-5.5}$.

[17] Regarding to observe the influence of the supernatant on the multiplication of the test virus, the challenged dermal parts were removed at different intervals, and subjected to the titration of virulence. [18] Active virus was not seen in any case. [19] Rarely, the dermal tissue showed, towards the forth day, a virulence of 10^{-1} to10^{-2} (see Figure 1).

Study to show the effect of the inhibiting factor. — [20] In the precedent report, it was shown that the partially purified and inactivated virus possesses an immunogenic strength just as well as an interference strength. [21] In order to know if the supernatant possesses a vaccinating strength, we practiced the following experiments: at different intervals after the injection of the supernatant, the rabbit received the test virus. [22] The most marked resistance was observed against the test practiced at the end of 24 hours. [23] In contrast to the case of the treatment with the purified virus, the skin did not withstand to the challenge at the 14th day (see Figure 2). [24] The supernatant seems to be therefore devoid of vaccinating strength.



●——● Supernatant of the brayed of non-infected tissue (dose of challenge:)

○- - - - - -○ Ibid. (dose d'épreuve : $10^{1.5}$ x DI_{50}.)

▲——▲ Inactivated purified virus (dose d'épreuve : 10^{3} x DI_{50}.)

△- - - - - -△ Ibid. (dose d'épreuve : $10^{1.5}$ x DI_{50}.)

■——■ Supernatant of the brayed of infected tissue (dose of challenge: 10^{3} x DI_{50}.)

□- - - - - -□ Ibid. (dose d'épreuve : $10^{1.5}$ x DI_{50}.)

Fig. 1. — Inhibition of the multiplication of the virus by the supernatant of the brayed of infected tissue.

[25] The supernatant should be able to contain a trace of neutralizing antibodies to inhibit the infection. [26] But, in reality, the inhibition by the supernatant shows more intensive against the test of the next day than against the immediate challenge, while the resistance conferred by the injection of the immune-serum presents the strongest against the test of the same day, and then lowers progressively. [27] The inhibition would not be attributed to the neutralizing antibodies that can be found at a slight quantity in the supernatant.

Production of the inhibitory factor. — [28] In the infected skin, the inhibitory factor appeared towards the 24 hours, and increased progressively until the 4th day. [29] The evolution of the production of the inhibitory factor thus is found parallel to that of the infectivity and antigen setting up the complement. [30] From the testicular materiel, we could prepare a supernatant with the inhibiting effect. [31] When we inoculated the chorio-allantoic membrane of the egg of the hen, the brayed membrane presented a virulence of $10^{9.2}$ and the positive reaction of the fixation of the complement even at 1: 640. [32] The supernatant of the brayed was found, nevertheless, lacking in the inhibition property.

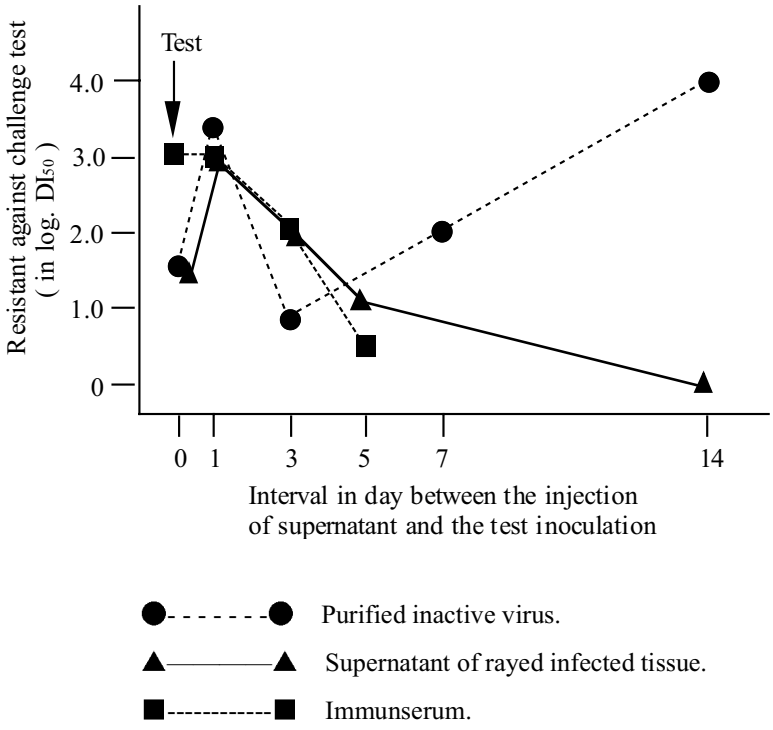


Fig. 2. — Optimum interval between the injection of the supernatants and the test inoculation.

Some physical properties of the inhibitory factor. — [33] A heating for 30 minutes at 56° C does not affect hardly the inhibitory effect of the supernatant. [34] Heated at 65° C,

the effect remarkably weakens. [35] In the conditions of our experiments, an ultraviolet irradiation for 20 minutes respects the inhibiting effect of the supernatant, while the effect of the purified virus purifies was totally removed by an irradiation for 12 minutes.
[36] The inhibiting factor in the supernatant is not precipitated by a centrifugation for two hours at 10^5 g. [37] The inhibiting principle crosses the filter of Seitz. [38] The factor is not dialyzed through the membrane of cellophane.

Resume. — [39] The non-infectious supernatant obtained by repeated centrifugation from the brayed dermal or testicular tissues of the rabbit infected by the vaccinia virus can inhibit the vaccinia infection of the rabbit skin. [40] It would be not a matter of immunity, neither actively nor passively. [41] In contrast to the virus particle, the inhibiting principle in the supernatant withstands the ultraviolet rays. [42] A centrifugation for two hours at 10^5 g does not precipitate the inhibiting principle. [43] It is not dialyzed through the membrane of cellophane.

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(1) Y. Nagana et S. Furuno, *Japan. J. exp. Med.*, 1951, t. *21*, p. 97 ; Y. Nagano, Y. Kojima et Y. Sawai, *C. R. Soc. Biol.*, 1954, t. *146*, p. 750 ; Y. Nagano et Y. Kojima, *C. R. Soc Biol.*, 1954, t. *148*, p. 1700 ; 1958, t. *152*, p 372.
(2) R. F. Parker et T. M. Rivers, *J. exp. Med.*, 1935, t. *62*, p. 65.

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