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<td>Author(s)</td>
<td>SUZUKI, Masatoshi</td>
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<tr>
<td>Citation</td>
<td>Japanese Journal of Veterinary Research, 16(2-3): 87-88</td>
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<tr>
<td>Issue Date</td>
<td>1968-09</td>
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<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/1902">http://hdl.handle.net/2115/1902</a></td>
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<tr>
<td>Type</td>
<td>bulletin</td>
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<tr>
<td>File Information</td>
<td>KJ00002369720.pdf</td>
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STUDIES ON FREEZE-DRYING OF VACCINIA VIRUS
EFFECT OF SUSPENDING MEDIA ON INFECTIVITY TITERS*

Masatoshi SUZUKI
Japan BCG Laboratory
Kiyose-machi, Kitamagun, Tokyo, Japan

The purpose of this study is to show the protective effects of several single and combined suspending media on the infectivity titers of vaccinia virus in the freeze-drying, and in the process of preservation after the freeze-drying.

Freeze-dried smallpox vaccine and purified dried smallpox vaccine, prepared from calf dermal pulp (CDP) or infected chorio-allantoic membrane (CAM) of the developing chicken embryo as vaccinia virus materials, were used in this study. Results may be summarized as follows.

1) As for the single suspending medium, sodium glutamate and peptone showed the protective effect on the infectivity titers in the dried vaccines from CDP and CAM, and in the purified dried vaccines from CDP and CAM. But lactose and glucose were not effective in the dried vaccine from CAM, and the former in the purified vaccine from CAM.

2) The effect of the concentration of sodium glutamate as a single medium was evident in the purified dried vaccines from CDP and CAM. It clarified an optimum concentration of sodium glutamate as a protectant. In the dried vaccine from CDP, the difference in the protective effect by the concentration of sodium glutamate was seen, when the vaccine was preserved at high temperature (45°C) for a long period, against both of the purified dried vaccines from CDP and CAM.

3) The combined suspending media were not effective in the dried vaccine from CDP. However, in the purified dried vaccines from CDP and CAM, these media revealed the protective effect, when the concentration of sodium glutamate for the first medium higher than the optimum concentration in the single medium

* Original paper of this article is submitted to Journal of Hygiene.

JAP. J. VET. RES., VOL. 16, NOS. 2 & 3, 1968
was used.

4) Moisture content (1.42 to 3.71%) of the dried vaccine from CDP, containing sodium glutamate as the single medium, did not influence the protective effect of the suspending medium, when the vaccine was exposed at high temperature (100°C, 60 minutes). In peptone, also, the relation between the protective effect of its medium and the moisture content (0.11 to 4.24%) was not shown.

5) In the preservation process at 45°C, the protective effect of sodium glutamate or peptone as the single medium in the dried vaccine from CDP was not affected by the moisture content from 0.65 to 4.07% in the former and 1.23 to 3.03% in the latter.

Hokkaido University granted the degree of Master of Veterinary Medicine to the following 8 graduates of the Post-Graduate School on March 25, 1968.

The authors’ summaries of their theses are as follows:

**A SEARCH FOR SUSCEPTIBLE CELLS TO AVIAN ENCEPHALOMYELITIS (AE) VIRUS**

Takemaru Abe

*Department of Epizootiology*

*Faculty of Veterinary Medicine*

*Hokkaido University, Sapporo, Japan*

(Summary of Masters thesis written under direction of Dr. S. Miura)

This report is a study of embryo-adapted avian encephalomyelitis (AE) virus was initiated to determine its cytopathogenic effect on various selected tissue cell cultures.

The following cell types were used in this study. (1) Primary cultures of the following chicken cells: glia cells, embryo whole brain cells, whole embryo fibro-blasts, embryonic cells of the heart, liver, kidney, lung, intestine and spleen. (2) Primary cultured kidney cells from the monkey, calf and pig. (3) Fifteen established cell lines: HeLa, HeLa S-3, FL, FL-17-M, E 10, E 38, 1035, HEp 2, G2, MS, MK2, Vero, L, JTC-5 and SK.

A summary of our results follows:

1) During the first seven days after inoculation with the AE virus (10^4.5 EID_{50}/0.1 ml), diluted to 10^0 to 10^5, no cytopathogenic effect (CPE) was observed in the above-mentioned 27 cell types.