COMMERCIAL CULTIVATION OF STERILE VACCINIA VIRUS

H. C. Allisbaugh and L. C. Morgan, Lilly Research Laboratories, Indianapolis

Since the time of Jenner, nearly 150 years ago, there has been a constant intensive search for a safe and improved virus for the preventive inoculation against small-pox. Vaccinia virus, grown on the calf, having a uniform high potency and a low count of bacterial contaminants, had been the reward until very recently.

Goodpasture and Buddingh (1) described a technique for the cultivation of sterile vaccinia virus on the chorioallantoic membrane of the developing chicken egg. Such a vaccine is free of all bacteria and their toxins. It can be produced easily and quickly, eliminating the necesstiy of long storage. Virus so grown on the developing egg membrane seems less likely to assume neuro-tropic properties than that grown on the calf.

Some modifications of the original technique were necessary for the cultivation of the virus in sufficiently large quantities for commercial distribution. Fertile eggs, not more than 48 hours old, are secured from a selected flock of chickens. Only eggs having well-formed shells are used. On receipt at the laboratory, these are incubated for twelve to fourteen days at 38° C., being turned daily. At the end of this time they are candled and the ones having well-developed embryos are inoculated with the seed virus.

Inoculation

Inoculation can be made by either of two methods.

A. *The shell flap method.* A triangle, one-half inch on a side, is cut through the shell using a small carborundum dental wheel. The shell membrane is cut with a scalpel. The shell flap is then turned back exposing the chorio-allantoic membrane. A small piece of virus-containing tissue is brushed gently over the exposed surface of the membrane. The shell is replaced and sealed with sterile melted paraffin.

B. *The Puncture Method.* A small hole is broken or drilled into the side of the shell, care being taken not to puncture the chorio-allantoic membrane. One-fiftieth of a cubic centimeter of a rich virus suspension is then introduced through the opening with a capillary pippette. The virus spreads over the exterior of the chorio-allantoic membrane. The seed virus used is suspended in 1% saline, since this diluent has a tendency to promote multiple lesions (Rao and Pandit, 2). The opening is sealed with sterile melted paraffin.

Harvesting

After a further incubation of four days, the infected membranes are harvested. The eggs inoculated by the first method are harvested by removing a large section of the shell, including the original flap, and exposing the infected membrane. The lesion surrounded by a border of the membrane is cut off and placed into a sterile Petri dish.

The eggs inoculated by the puncture method are harvested by breaking out the shell over the air-cell, removing the air-cell membrane,

and taking out the embryo. The whole chorio-allantoic membrane is then removed from the shell and placed in a sterile Petri dish.

Aseptic technique must be observed throughout the whole procedure. The membranes in each Petri dish are sterility tested on broth fermentation tubes and agar slants, then covered with 50% glycerin containing 1:10,000 Merthiolate.

Grinding

The membranes found to be sterile are aseptically pooled in a large mortar packed in dry ice. While frozen solid they are ground to a fine powder. The desired amount of 50% glycerin is added to the powder and the mixture again tested for sterility on broth fermentation tubes, agar slants and agar pour plates. The potency is determined by serial dilution and inoculation on rabbits. The safety of the product is tested by intra-peritoneal inoculation of mice and guinea pigs.

Filling

The ground lot, if shown to be sterile, is filled and sealed in capillary tubes. The sterility is again tested on broth fermentation tubes, agar slants and agar pour plates. The potency and safety of the virus is again checked by animal inoculation. After the product has successfully passed these rigid tests, it is finished, packaged, and distributed for human immunization.

Discussion

The potency of the average lot of chorio-allantoic vaccinia virus gives a confluent take, on the skin of a shaven rabbit, in a dilution of 1:1000. Any lot which falls below this level is discarded. Passage of the virus in the egg seems to maintain its potency at a uniform level indefinitely without intermediate animal passage. The thermostability of the virus at room temperature or in the refrigerator equals that of calf vaccine virus.

The lesions in human inoculations have been uniformly less severe than those obtained with calf virus. The lesion develops normally but produces a thinner, more superficial scar. Data gathered after several months of production clearly indicate that this vaccine is comparable to the calf vaccine both in primary inoculations and degree of immunity established.

Conclusion

The cultivation of vaccinia virus on the chorio-allantoic membrane of the developing egg, makes possible a sterile immunizing agent of uniform high potency.

References

1. Goodpasture, E. W., and G. John Buddingh, 1935. Am. Jour. of Hyg. 21:319-360.

2. Pandit, C. G., and R. S. Rao, 1935. Bull. Office. Internat. d'Hyg. Pub. 27:53-66.