Loss of Hemagglutinin on Passage of Vaccinia Virus in L Cells.*† (24723)

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When vaccinia virus was passed serially through Ehrlich ascites tumor, it lost ability to produce hemagglutinin (HA) in embryonated eggs, and capacity to induce formation of HA inhibiting antibodies in rabbits(1,2). Presence of a nonspecific inhibitor of vaccinia HA in cell-free plasma of the Ehrlich tumor (the Ehrlich inhibitor)(3) suggested that this inhibitor might be directly involved in the change of hemagglutinating vaccinia virus to the type of virus which lacks capacity to produce HA. The current experiments concerned with serial passage of vaccinia virus in tissue cultures of L cells strongly indicate the probability of such a situation.

Materials and methods. Virus. Virus designated IHD-E is a neurotropic line of the IHD strain of vaccinia virus, while virus designated IHD-23T is a subline of IHD-E obtained following 23 passages in the Ehrlich tumor. IHD-E produces HA in embryonated eggs, but IHD-23T does not(1). Methods of assaying virus and preparing suspensions for HA and HA-inhibition titrations have been described(1,3,4). *Ehrlich inhibitor.* Cell-free fluids from female NIH mice bearing 13-day Ehrlich ascites tumors (Lettré hyperdiploid line with modal count of 45 chromosomes/cell) served as source of inhibitor. Prior to use, the fluids were subjected to 4 high speed cycles of centrifugation with the fluid decanted from tubes at each cycle. The pooled fluid was then cultured to assure freedom from bacterial contamination, and was stored for a minimum of 6 weeks at –27°C before use(5). When this fluid was thawed for use it received a final centrifugation. Only those fluids showing HA-inhibition titers of 1:80 or greater were employed in tissue culture studies described below. *Tissue culture.* Cultures of the L cell(6,7) were carried in 160 ml pyrex milk-dilution bottles, inoculated at weekly intervals with 1 x 10^6 cells/bottle. The medium consisted of 8.5 ml of medium 199(8) and 1.5 ml of horse serum/bottle. Before use, the horse serum was filtered through Millipore disc, and then heated at 56°C for 30 minutes. Since a nonspecific, heat labile inhibitor was found in certain lots of horse serum, these were carefully avoided.

Virus was passed serially in the medium described above. In addition, virus was passed serially in a medium containing Ehrlich inhibitor. This consisted of 1 ml of a 10X solution of medium 199, 8.5 ml of ascitic fluid containing the Ehrlich inhibitor, and 0.5 ml of horse serum. Five million cells were planted in each bottle, and 4 hours later the virus was inoculated. pH of various preparations was adjusted with phenol red-bicarbonate solution, antibiotics were added to give a final concentration of 200 units of penicillin and 100 μg of streptomycin/ml, and cultures were incubated at 37°C. Control cultures of L cells to which no vaccinia virus was added, but which contained the Ehrlich inhibitor, were employed to eliminate the possibility of contamination with vaccinia virus. Titer of Ehrlich inhibitor in these controls was reduced to one half its original value when the cultures were 6 days old.

Results. When cultures of the L cell in either horse serum medium or Ehrlich inhibitor medium were inoculated with 2 x 10^2 pock-producing particles of virus (ppp), an infectivity peak was reached in 6 days. The titer at this time showed more than a thousand-fold increase in infectious virus. As a matter of convenience, fluids from each virus passage in L cells were harvested at 7 days. Approximately 2 x 10^3 ppp were used to inoculate the L cells in each succeeding passage. From Table I it is apparent that the IHD-E strain of vaccinia virus loses ability to produce HA in embryonated eggs following 17 serial passages in L cells in presence of Ehr-

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TABLE I. Serial Passage of Vaccinia Virus in Tissue Cultures of L Cells.

Serial TC passage	HA titer* Passage in horse serum Virus IHD-E	Virus IHD-23T	Passage in Ehrlich inhibitor Virus IHD-E	Virus IHD-23T
5	40	0†	40	0
10	"	0	80	0
15	"	0	20	0
16			20	
17			0	
18			0	
19			0	
20	80	0	0	0
25	160	0	0	0
30	80	0	0	0

* Hemagglutinin titers of 20% suspensions of chorioallantoic membranes which had been inoculated with 1:10 dilutions of virus-containing tissue culture fluids.

† Designation 0 indicates no hemagglutination at a 1:10 dilution of membrane suspension.

lich inhibitor. In another experiment the hemagglutinating property of the IHD-E virus, passed in L cells in the presence of the inhibitor, was lost after 20 passages.

The results recorded in Table II show that ability to recover the hemagglutinating type of virus is dependent upon number of passages which IHD-E virus has had in L cells in the presence of Ehrlich inhibitor. After 17 tissue culture passages, virus with capacity to produce HA can be recovered after 2 passages in embryonated eggs. On the other hand, after 24 tissue culture passages, the hemagglutinating type of virus could not be recovered after 31 egg passages. The data suggest that the virus with ability to produce HA is gradually replaced by virus which cannot form HA. IHD-E passed in L cells in horse serum medium retains hemagglutinating activity, while IHD-23T retains its inability to produce HA when passed in horse serum or in the Ehrlich inhibitor.

TABLE II. Recovery of Hemagglutinin on Serial Passage of Vaccinia Virus in Embryonated Eggs.

Virus	No. of egg passages required to recover HA
IHD-E 17TC	2
18TC	7
19TC	7
20TC	17
21TC	17
22TC	28
23TC	28
24TC	Not recovered after 31 passages
25TC	*Idem*
26TC	"
27TC	"
28TC	"
29TC	"
30TC	"

Summary. Cell-free ascitic fluids obtained from mice bearing Ehrlich tumor contain an inhibitor which prevents vaccinia virus hemagglutination. Serial passage of the virus in tissue cultures of L cells in presence of the inhibitor favors appearance of virus which has lost ability to produce hemagglutinin. Serial passage of the virus under comparable conditions in the absence of inhibitor did not influence hemagglutinin-producing activity of the virus. These results support the assumption that the loss of hemagglutinin-producing ability by vaccinia virus, on passage through the Ehrlich tumor, results from action of the inhibitor.

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