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Preliminary Report

Detection of Hemagglutinin Production of Vaccinia Virus by Hemadsorption

There are many studies on the hemagglutinin of vaccinia virus (Nagler, 1942; Burnet, 1946; Stone and Burnet, 1946). In this paper, however, the author wishes to describe a method by which hemagglutinin production with vaccinia virus can be detected on monolayers of L cells by the hemadsorption technique. The IHD strains of vaccinia virus used for this work were HA^+ and HA^- substrains. Although the former was able to produce the hemagglutinin, the latter was not. HA^+ virus had been passaged directly in L cells, whereas HA^- virus had been passaged ten times in Ehrlich's ascites tumor cells and then in L cells, as reported by Cassel and Fater (1958).

Viral samples were obtained by freezing and thawing of infected L cells and centrifugation to remove cellular debris. L cell monolayers for morphological observation of infected cells, were prepared in 10 ml square bottles or on 10×30 mm coverslips in square bottles. The culture medium was a mixture of 95 parts YLH solution, which consists of Hank's balanced salt solution containing 0.5 per cent lactalbumin hydrolyzate and 0.1 per cent yeast extract, and 5 parts bovine serum. For plating of virus, the culture medium was removed from the bottle. Aliquots of 0.2 ml of viral samples ($5.0 \times 10^7 TCID_{50}$) were inoculated into the bottles and incubated at 37°C. After 2 hours, the infected cells were washed twice with YLH solution and then mixed with the culture medium containing 99 parts YLH solution and 1 part calf serum. For hemadsorption, the culture medium was removed from the bottle, and after washing with phosphate buffered saline, the monolayers were covered with a 0.2 per cent suspension of chicken red blood cells in phosphate buffered saline for 30 minutes at room temperature. After allowing hemadsorption, the monolayers were washed twice with phosphate buffered saline. The coverslips were taken out from the bottles, fixed with methanol, and then stained with Giemsa solution.

Figs. 1, 2, and 3 show the hemadsorption of chicken red blood cells around L cells infected with HA^+ virus. Fig. 4 shows nonhemadsorptive L cells infected with HA^- virus. Table 1 shows the relations between hemadsorption, "B" type inclusion bodies, and giant cell formation. The appearance of hemadsorption in L cells infected with HA^+ virus begins 10 hours after infection and the maximum hemadsorption is observed 24 hours after viral inoculation. The appearance of "B" type inclusion bodies (Kato *et al.*, 1959, 1960) and hemadsorption either seem to occur at about the same time or the formation of "B" type inclusion bodies seems to occur slightly earlier than the beginning of hemadsorption. Kim (1958) reported that the hemagglutinin in the ectromelia infected Ehrlich tumor cells first became appreciable 12 hours after infection and a fair parallelism was found between the growth of inculusion bodies and the production of hemagglutinin. The

Table 1. Relations between Hemadsorption, "B" type Inclusion bodies, and Giant Cell Formation

Hours after infection	HA^+ vaccinia virus			HA^- vaccinia virus		
	hemadsorption	"B" type inclusion bodies	giant cell formation	hemadsorption	"B" type inclution bodies	giant cell formation
4	—	—	—	—	—	—
8	—	—	—	—	—	—
10	+	+	—	—	+	—
12	+	+	—	—	+	—
24	+	+	+	—	+	+
48	+	+	+	—	+	+

development of giant cells which contain a number of nuclei was seen after the beginning of hemadsorption of chiken red blood cells and the appearance of "B" type inclusion bodies.

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EXPLANATION OF PHOTOGRAPHS

Fig. 1 Hemadsorption of chicken red blood cells around L cells infected with HA^+ vaccinia virus. ×100

Fig. 2 Hemadsorption of chicken red blood cells around L cells infected with HA^+ vaccinia virus. Giemsa staing. ×400

Hemadsorption and "B" type inclusion bodies are seen.

Figs. 3 and 4 Hemadsorption and nonhemadsorption of chicken red blood cells around L cells infected with HA^+ and HA^- vaccinia virus respectively. Giemsa staining. ×100

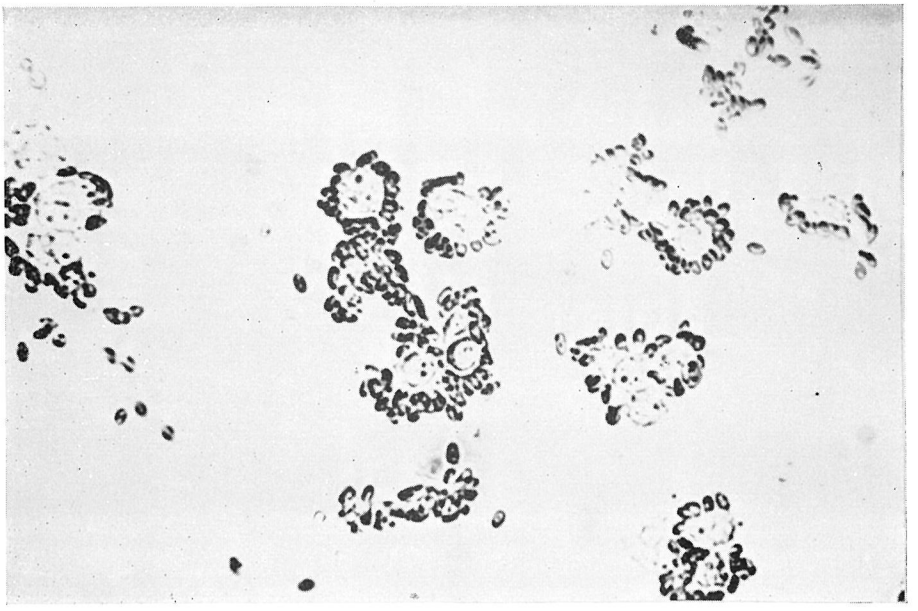

Fig. 1.

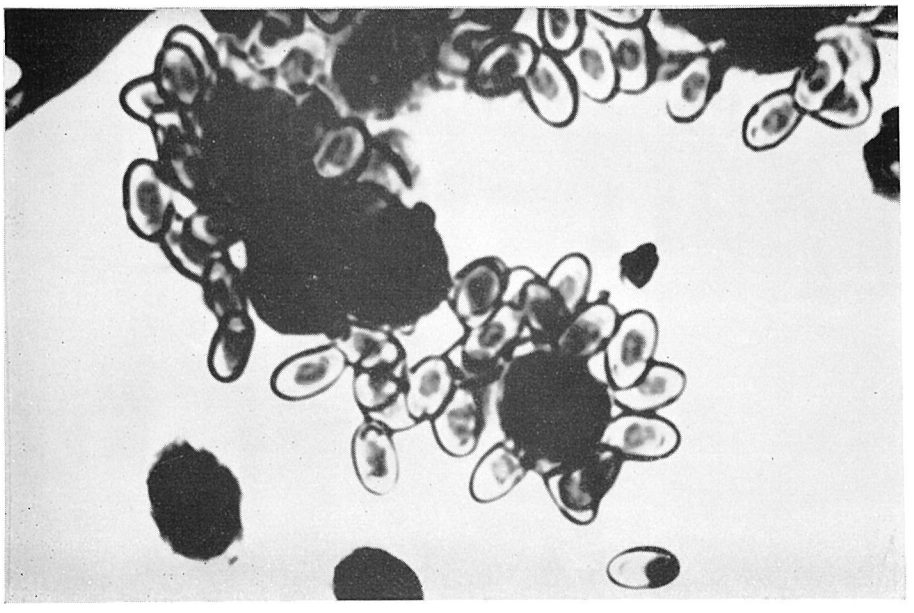

Fig. 2.

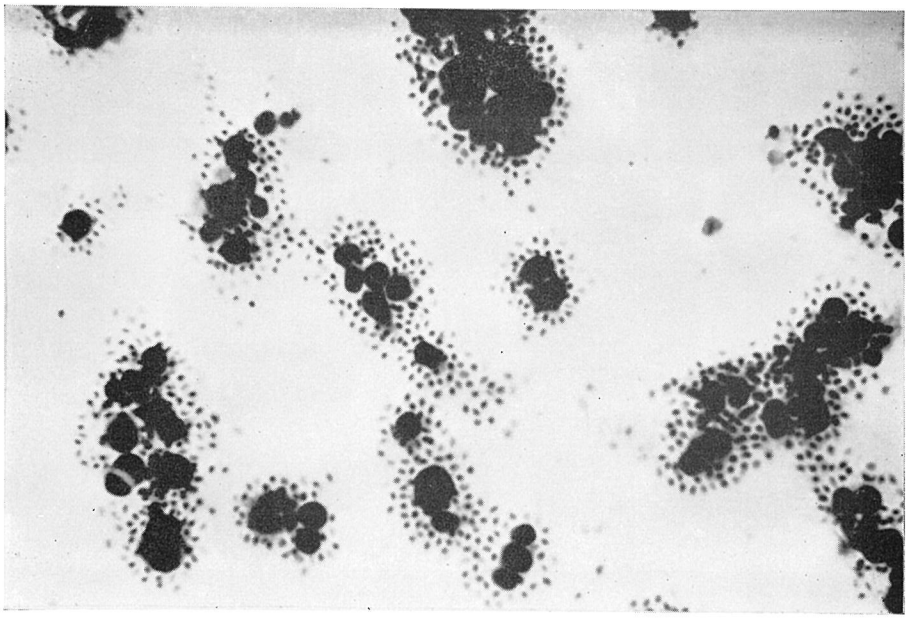

Fig. 3.

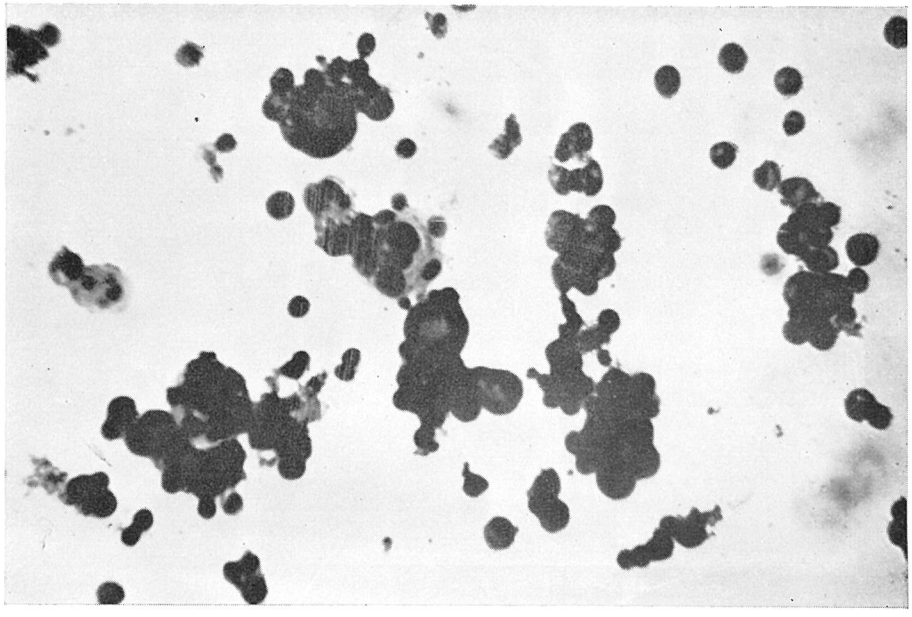

Fig. 4.