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Enhancement of Vaccinia Virus Plaque Formation by Trypsin.* (32492)

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The purpose of this report is to describe a phenomenon which was observed when we attempted to characterize a viral inhibitor. Trypsin was found greatly to enhance virus plaque formation when it was included in the medium.

Materials and methods. Media and cell cultures. Growth medium for the establishment of chick cell cultures consisted of Gey's balanced salt solution (BSS) with 5% calf serum, 0.1% lactalbumin hydrolysate, 0.1% proteose peptone, and 0.06% sodium bicarbonate. Maintenance medium used for virus assays with vaccinia virus consisted of BSS with 0.11% sodium bicarbonate, 0.1% proteose peptone, 0.1% lactalbumin hydrolysate, and 0.1% yeast extract. Chick embryo cell cultures were prepared as previously described (1).

Virus. Vaccinia virus, strain NY 914, was grown on the chorioallantois of 10 to 11-day-old developing chick embryos. Infected membranes were removed 48 hours after inoculation and triturated with maintenance medium. Virus preparations were centrifuged at 800 *g* for 30 minutes to remove cellular debris and were stored in glass ampules at −60°. Virus was assayed according to the method described by Lindenmann and Gifford(2).

Trypsin and soybean trypsin inhibitor. Trypsin, 2.5% solution, was obtained from the Grand Island Biological Co., Grand Island, N. Y. Crystalline soybean trypsin inhibitor was obtained from Mann Research Laboratories, New York. Both substances were diluted in maintenance medium.

Results. Trypsin was added to a preparation of a viral inhibitor in order to study its susceptibility to this enzyme. After a period of incubation soybean trypsin inhibitor in approximately equimolar concentrations was added and residual antiviral activity was measured in chick embryo cultures challenged with vaccinia virus by the method of Lindenmann and Gifford(2). Surprisingly, the numbers and size of vaccinia plaques which developed over the next 48 hours were greatly increased when compared to control cultures. The phenomenon was further studied since it increased the efficiency of quantifying this virus and may have applications to other viruses. The antiviral agent originally employed was found to be irrelevant in demon-

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TABLE I.

Additions	Plaque No.	Avg plaque size* (mm^2)	Total plaque area* (mm^2)	Relative to control cultures		
				No.	avg size	total area
none	348	15.6	5440	—	—	—
.1 mg trypsin	569	26.6	15130	1.6	1.7	2.8
.2 " " †	517	101.2	52340	1.5	6.5	9.6
.5 " " + .5 mg SBTI‡	505	112.2	56650	1.5	7.2	10.4
1.0 mg trypsin + 1.0 mg SBTI	495	108.5	53680	1.4	7.0	9.9

* Sizes are measured in mm on drawings made after magnification of monolayers 6.5 × with a photographic enlarger.

† Concentrations of trypsin greater than 0.2 mg caused detachment of cells from the glass.

‡ SBTI = soybean trypsin inhibitor.

strating the phenomenon and was omitted from further studies.

The possibility existed that insufficient soybean trypsin inhibitor was added completely to inhibit trypsin activity(3) although the cells did not become detached from the glass. The phenomenon was further studied using trypsin and mixtures of trypsin and soybean trypsin inhibitor. Soybean trypsin inhibitor alone had no effect on vaccinia virus plaque formation. The results of one such study are shown in Table I. Vaccinia virus was diluted in maintenance medium which did not contain serum and therefore natural trypsin inhibitors were not present in the medium. Virus suspensions were added to well-drained cultures of chick embryo cell monolayers and trypsin and soybean trypsin inhibitor were added at concentrations indicated in the table and the final volume adjusted to 2.0 ml. Cultures were incubated at 37° for 48 hours, the medium decanted, and the monolayers stained with crystal violet. Plaques were drawn, measured, and counted after projection with a photographic enlarger giving 6.5 × magnification. As can be seen from Table I,

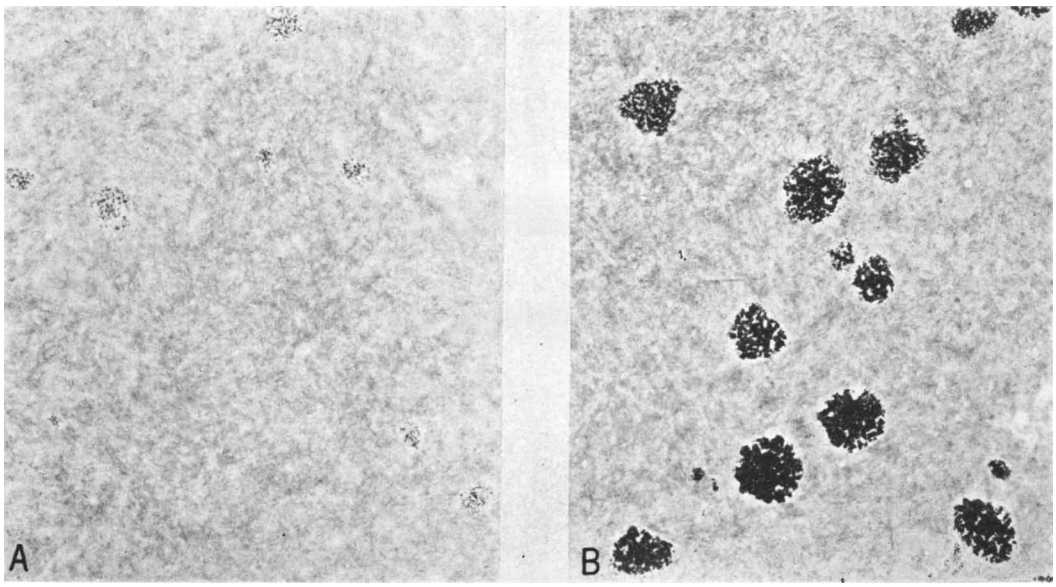

FIG. 1. Plaques of vaccinia virus in absence (A) and presence (B) of trypsin. The stained cell sheets were used as "negatives" in a photographic enlarger. Magnification of 6.5 ×.

the presence of trypsin in the supernatant fluid of such infected cultures resulted in approximately 1.5 times more plaques than in the control cultures. In addition, the plaque size was markedly increased and the average area was 6.5 times larger in the presence of the greatest amount of trypsin which could be employed without detaching the cells from the glass. Higher concentrations of trypsin could be employed in the presence of soybean trypsin inhibitor but such combinations did not have any obvious advantage. Fig. 1 shows enlarged prints of the plaques 48 hours after infection in the presence or absence of trypsin, the stained monolayers being used as "negatives" in a photographic enlarger. Plaques are also more distinct in the presence of trypsin and contain less debris than in the control bottles. From drawings of a large number of magnified plaques observed in these studies, histograms of plaque size frequencies were established (Fig. 2).

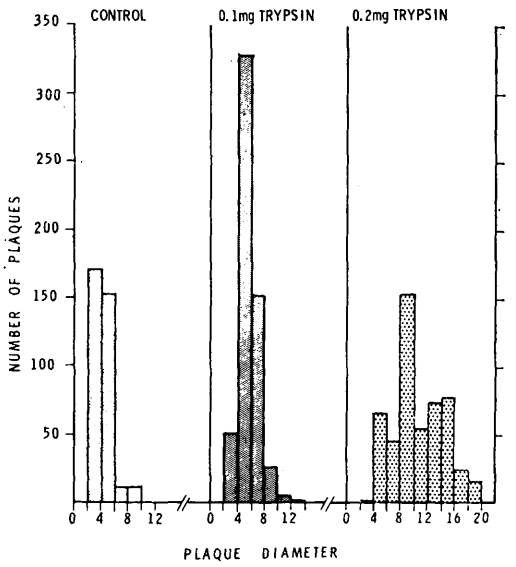

FIG. 2. Histograms of plaque diameters 48 hr after infection. Abscissa: Diameter of 6.5 times magnified plaques in millimeters.

Discussion. It is obvious that vaccinia virus plaque formation is enhanced by the presence of trypsin in the fluid overlay. The addition of this one substance greatly enhances the plating efficiency of this virus and the larger plaque size simplifies the scoring of plaques. The increased rate of growth of plaques in the presence of trypsin may cause plaques hidden below the threshold of visibility ("infraplaques") to become visible and thereby increase the number of plaque forming units that can be scored. The reason for the enhanced rate of growth is not understood. Plaque formation can be expected to depend upon the interaction of a number of factors (4). It is possible that virus-cell interactions themselves could contribute to limitations of plaque growth through local accumulation of interferon and that trypsin destroys this interferon. The infectivity of reovirus has been shown to be enhanced by proteolytic enzymes (5,6).

Summary. The size and numbers of vaccinia plaques in chick embryo cell cultures are enhanced by trypsin.

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