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Plaque Assay for Measurement of Cells Infected with Zoster Virus

The isolation of viruses belonging to the varicella-zoster group in human cells by Weller and his associates (*1*, *2*) led to attempts to study the properties of these viruses by conventional methods. However, viruses in this group have proved to be difficult to grow and can be propagated only when intact infected cells are used as inoculum. A recent isolate of zoster virus (strain EY) in our laboratory has the same characteristics as the strains previously isolated by others. The virus was isolated in human embryonic lung fibroblasts from fresh vesicular fluid from a patient with generalized zoster infection. Many attempts to detect cell-free virus in the extracellular fluids were negative. This was true even when the cultures were actively deteriorating. The virus has therefore been passed by adding dispersed cells from infected cultures to cell suspensions or monolayers of human embryonic lung cells. Stocks of virus are maintained by freezing infected cells in Eagle's medium with 20-percent calf serum and 15-percent glycerol in liquid nitrogen at −195°C, under which condition the virus-cell complex retains its infectivity.

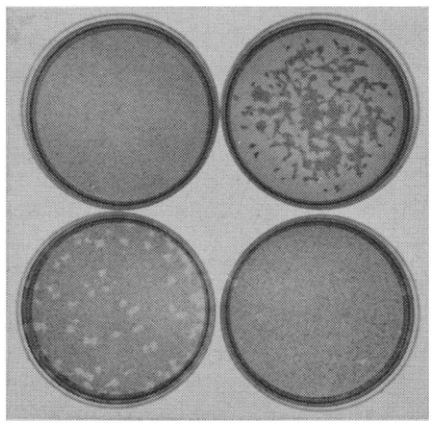

Fig. 1. Plaques obtained under an agar overlay 10 days after inoculation of human embryonic lung fibroblasts with cells infected with zoster virus. (Upper left) uninoculated. (Upper right) dilution of 10^{-2}. (Lower left) dilution of 10^{-3}. (Lower right) dilution of 10^{-4}. (× 0.4)

Infected cultures exhibit focal cytopathic effects which become manifest throughout the culture. This spread of the effect is slower than that exhibited in human embryonic lung cells by herpes simplex virus (24 to 48 hours to complete degeneration) but more rapid than that seen with cytomegalovirus (5 to 6 days) when tested under the same conditions.

Another feature that distinguishes this isolate from cytomegalovirus and herpes simplex virus is its lack of viability when infected cells are disrupted. Thus, freezing and thawing or sonic oscillation of infected cells causes complete destruction of the infectivity of the virus. Herpes simplex virus survives both these procedures, and we have found that cytomegalovirus can be liberated from infected cells by sonic oscillation.

Serums from convalescent patients with zoster or varicella infection react with virus antigen produced in these cells as measured by immunofluorescence, but specific herpes simplex antiserums prepared in rabbits fail to do so. Serums from very young children without previous exposure to varicella often react with cytomegaloantigen but do not react with zoster antigen.

During the course of these studies, it was noted that cultures inoculated with small numbers of infected cells exhibited distinct circumscribed areas of degeneration with clear centers. This has also been described by Weller *et al.* (*2*) and by Taylor-Robinson (*3*). Attempts to obtain a quantitative plaque assay without an overlay, however, were unsuccessful because of the rapid spread of the infection under the fluid medium; this was probably mediated by detached cells. An attempt was therefore made to develop a plaque assay system for cells infected with zoster virus under a semisolid overlay. Infected human fibroblasts in which 50 to 75 percent of the cells showed some measure of cytopathic effect were trypsinized with 0.2 percent trypsin, resuspended in growth medium consisting of 90-percent Eagle's medium and 10-percent calf serum, and adjusted to a concentration of 2×10^7 cells per milliliter. The cells were then diluted in nutrient medium and plated out in either 0.5 or 1.0 $\log_{10}$ dilutions on monolayers of human fibroblasts growing in 60-mm petri dishes.

Table 1. Plaque assay of cells infected with zoster virus; read 10 days after inoculation when plaques ranged from 2 to 3 mm in diameter. PFC, plaque-forming cells.

Dilution	Plaque counts	Av.	PFC per ml
10^{-3}	*		
10^{-4}	33, 36, 36, 42	36.3	3.6×10^6
10^{-5}	4, 2, 2, 7	3.8	3.8×10^6
$10^{-3.5}$	*		
10^{-4}	58, 60	59	5.9×10^6
$10^{-4.5}$	26, 22	24	7.6×10^6
10^{-5}	6, 6	6	6.0×10^6

* Too numerous to count in two to four plates used at dilution indicated.

After an adsorption period that varied from 3 to 18 hours, the nutrient fluid was withdrawn and an overlay that consisted of either 1 percent agar or 2 percent methyl cellulose (4000 cp) in Eagle's basal medium plus 10-percent calf serum was added to the petri dishes. The cultures were then incubated at 37°C in an atmosphere of 5-percent CO_2 for varying periods of time. The day before plaques were to be examined, 3 ml of a 1 : 7500 concentration of neutral red was added to the cultures and the cultures were reincubated overnight at 37°C in 5-percent CO_2.

Plaques developed between 4 and 7 days after inoculation of the cells.

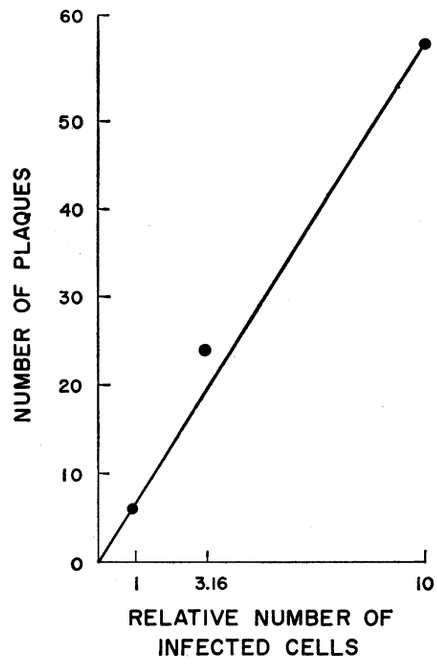

Fig. 2. Linear relationship between number of cells infected with zoster virus on monolayers of human embryonic lung fibroblasts and plaques obtained under an agar overlay 10 days after infection.

Table 2. Comparison of agar and methyl cellulose as overlay; read 7 days after inoculation when plaques were 1 to 2 mm in diameter. PFC, plaque-forming cells.

Dilution	Plaque counts	Av.	PFC per ml
Methyl cellulose as overlay			
$10^{-3.5}$	*		
10^{-4}	42, 39	40.5	4.1×10^6
$10^{-4.5}$	22, 15	18.5	5.8×10^6
10^{-5}	5, 8	6.5	6.5×10^6
Agar as overlay			
$10^{-3.5}$	*		
10^{-4}	36, 47	41.5	4.2×10^6
$10^{-4.5}$	15, 22	18.5	5.8×10^6
10^{-5}	6, 6	6	6.0×10^6

* Too numerous to count in the two plates used at dilutions indicated.

These grew in size from 1 to 2 mm to 2 to 3 mm between the 7th and 10th days after inoculation (Fig. 1) but the counts remained relatively constant. A linear relationship between the concentration of cells inoculated and number of plaques observed was obtained (Table 1 and Fig. 2). The number of plaques counted represented 10 to 33 percent of the total cells (infected plus noninfected) seeded onto the monolayers. The plaques observed under agar became larger and were somewhat easier to count than the plaques seen under methyl cellulose. The counts were comparable, however (Table 2). This is in contrast to other experiments performed in this laboratory with herpes simplex virus where agar appears to inhibit plaque formation. Routine plaque counts are now being carried out 7 to 10 days after inoculation. Incorporation of 5-iodo-2-deoxyuridine completely inhibited the formation of plaques by cells infected with zoster virus. Serum from convalescent patients with varicella infection incorporated in the overlay did not inhibit plaque formation, although the serum contained zoster antibodies as measured by the immunofluorescent technique.

Under the same conditions cytomegalovirus-infected cells have not formed plaques; therefore, the technique described here may be useful in distinguishing these closely related viruses. The technique may also prove useful for other viruses for which difficulty is encountered in preparing cell-free extracts. Studies are now under way to determine the kinetics of the spread of zoster virus in human fibroblasts, to study plaque-purified lines of infected cells, and to obtain cell-free viable virus (*4*).

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Division of Labor among Primitively Social Bees

Abstract. *Australian bees of the genera* Allodapula *and* Exoneura *which commonly live in colonies of two to several individuals exhibit a division of labor among the adult females. Most foraging individuals are unfertilized and have slender ovaries, yet gather pollen and carry it to the nest; in contrast, fertilized egg-laying bees are not commonly foragers. Such castes are functionally similar to workers and queens found in some members of two other families of bees and represent a noteworthy example of physiological and behavioral parallelism in which the activities of different individuals are coordinated to form a functional unit.*

Among well known social bees (*Apis, Trigona, Melipona, Bombus,* all in the family Apidae) it is obvious that a division of labor exists between the female castes; the queens lay most of the eggs and the workers perform most other functions in the colony. Less extreme but similar division of labor among females occurs in some groups of a wholly unrelated family, the sweat bees or Halictidae (*1*) which usually live in colonies of very few (often less than five) individuals. In this family external morphological differences between the castes are absent or negligible. In the majority of bees there is no cooperative activity in nest construction or provisioning and no division of labor; each female makes and provisions her own cells, usually, but not always (*2*), in an independent nest. A small group of genera of the family Xylocopidae (*Allodape, Exoneura,* and their relatives) has long been known to care for larvae progressively and in this group two or more females are sometimes found in a single nest (*3*). It therefore seemed desirable to learn if there are workerlike females showing cooperative activity in this group as well as in Apidae and Halictidae.

Table 1. Ovarian and spermathecal conditions of females collecting pollen.

Individuals observed	Spermatheca without sperms	Ovaries slender	Month
	Allodapula spp. (*5*)		
24	22	14	Various
	*Exoneura bicolor**		
22	19	20	November
	Exoneura variabilis †		
26	21	25	February
	Exoneura robusta †		
3	1	3	October
	Exoneura hackeri †		
2	0	2	December

* From the New England highlands of New South Wales. † From localities in southeastern Queensland.

In nonsocial bees, or any kinds of bees in which there are no workers and each female makes and provisions her own cells, females collecting pollen from flowers have enlarged ovaries and sperm cells in the spermathecae; as soon as she has provisioned a cell, such a bee lays an egg in it. Pollen collecting and transport by bees with slender ovaries is evidence of worker-like activities, especially if the bees are also unfertilized.

Data based on studies of Australian species of *Allodapula* and *Exoneura* (Table 1) show that a high percentage of the pollen-collecting individuals are unfertilized and do not have enlarged ovaries. These data indicate not only the activity of workers in foraging but the inactivity of queens. Studies of nests of *Exoneura variabilis* showed that pollen was being carried into the nests and was presumably fed to larvae by workers. When such bees were present in a nest, the queen was not seen to carry pollen. Sometimes there was only one worker in a nest, in addition to the egg layer. In other nests two or three probable workers were recognized on dissection. No external morphological differences between egg layers and workers were found.

In these xylocopids, as in many halictids, a division of labor has arisen even in the very small colonies in which they live. Seemingly in all bees whose