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Isolations in a Mosquito (*Aedes pseudoscutellaris*) Cell Line (Mos. 61) of Yellow Fever Virus Strains from Original Field Material

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Summary. A simple, rapid and inexpensive method of isolating yellow fever (YF) virus from naturally infected mosquitoes, human liver and the serum of a sentinel monkey by inoculation of a continuous line of mosquito cells is described. The mosquito cells were more sensitive than suckling mice and marginally better than Vero cells for primary isolation. This is the first time that mosquito cells have been successfully used for primary isolation of YF virus from field material.

Although many cell lines have been established from mosquitoes and tested for their susceptibility to arboviruses (for a review, see Singh [1]), only three, one from *Aedes albopictus* [2–6; Dhandawate, quoted in 1] and the others from *Aedes malayensis* and *Aedes pseudoscutellaris* [7], have shown a cytopathic response when infected with at least some arboviruses.

Most of the work with mosquito cell lines has been done with mouse-adapted or mouse-passaged strains of arboviruses. There has been relatively little work on the infection and/or the cytopathic response of the cell lines to unadapted strains, i.e., original field material of naturally infected arthropod or vertebrate specimens. Singh and Paul [8] used the *A. albopictus* cell line for the successful primary isolation of dengue (DEN) virus from infected

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human serum and from *A. aegypti* mosquitoes infected in the laboratory with DEN-2 human serum. CHAPPELL *et al.* [9] found that the *A. albopictus* cells were better than newborn mice for isolating DEN-2 virus from human serum and naturally infected mosquitoes. VARMA *et al.* [7] reported that their *A. pseudoscutellaris* cell line (Mos. 61) also could be used for primary isolation of DEN-2 virus from human serum. SWEET and UNTHANK [6] observed that St. Louis encephalitis (SLE) virus in a known positive infectious mosquito pool produced a cytopathic effect (CPE) and a high yield of virus in the *A. albopictus* cell line.

We report below the successful use of the *A. pseudoscutellaris* cell line for primary isolation of yellow fever (YF) virus strains from pools of naturally infected mosquitoes, from human liver and from the serum of a sentinel monkey, and compare the results with those obtained independently for the same specimens in other isolation systems in the laboratory of origin.

Materials and Methods

Cells from the *Aedes pseudoscutellaris* (Mos. 61) cell line established by VARMA *et al.* [7] were used at subculture levels 135–140. Cultures were grown in 25-cm² plastic (Falcon) flasks in modified Liebovitz L-15 medium [7] with antibiotics using 10% fetal calf serum (FCS) for growth and 2% FCS for maintenance.

The five YF-infected field samples, two mosquito *(Haemagogus lucifer)* suspensions, two human liver suspensions and one sentinel rhesus monkey serum used in the isolation experiments are listed in table I and were received frozen over dry ice, identified only by code numbers. They were thawed and serial tenfold dilutions made in maintenance medium, the original material being treated as undiluted. Two flask cell cultures were inoculated with 0.4 ml of each dilution. Infected cultures were examined every day for 14 days for CPE. The same five field samples had previously been tested by one of us (P.H.P.) and by Mr. CLAYTON AJELLO of the Yale Arbovirus Research Unit using a second line of mosquito cells from *Aedes albopictus*, a line of monkey kidney (Vero) cells and suckling mice inoculated intracerebrally.

The titers are expressed as dex [10], the decimal exponent, per ml for 50% cytopathic dose (CPD_{50}) for cells or LD_{50} for suckling mice.

Results

The results are summarized in table I. Results obtained by one of us (P.H.P.) and by Mr. CLAYTON AJELLO (C.A.) with the same five field samples using *A. albopictus* cells, Vero cells and suckling mouse intracerebral inoculation are given for comparison. All five samples produced a distinct CPE

Table I. Results of isolation of YF virus from original field samples, in *Aedes pseudoscutellaris* (Mos. 61), *Aedes albopictus* and Vero cell cultures and in suckling mice

Original sample (No., description)	*Aedes pseudoscutellaris* (Mos. 61) cell cultures March–April 1975		*Aedes albopictus* cell cultures C.A., February 1975	Vero cell cultures				Suckling mouse intracerebral inoculation			
				P.H.P., August 1974		C.A., February 1975		P.H.P., August 1974		C.A., February 1975	
	CPD_{50} dex/ml	day p.i. (endpoint dilution)[a]	ID_{50} dex/ml[b]	CPD_{50} dex/ml	day p.i. (endpoint dilution)[a]	CPD_{50} dex/ml	day p.i. (endpoint dilution)[a]	LD_{50} dex/ml	day p.i. (endpoint dilution)[a]	LD_{50} dex/ml	day p.i. (endpoint dilution)[a]
303165, *Haemagogus lucifer* mosquitoes, pool 000772, 35 mosq. in 3 ml	4.9	6, 10 (10^{-4})	4.0	n.d.[c]	–	4.5	11, 12 (10^{-3})	n.d.	–	4.0	12 (10^{-3})
303547, *H. lucifer* mosquitoes, pool 000849, 30 mosq. in 3 ml	3.9	≤ 7 (10^{-3})	2.5	n.d.	–	4.5	12 (10^{-4})	n.d.	–	4.1	12 (10^{-3})
311376, 10% human liver suspension (case J.R.)	1.9	7 (10^{-2})	≤ 0.5[d]	2.6[e]	11 (10^{-2})	2.3[f]	9, 15 (10^{-1})	≤ 1.2[d]	–	$\simeq 1.3$[g]	12 (10^{-1})
311584, 10% human liver suspension (case P.S.)	1.9	9, 13 (10^{-1})	≤ 0.5[d]	2.0[h]	12–13 (10^{-1})	≤ 0.5[d]	–	≤ 1.2[d]	–	≤ 1.2[d]	–
902677, sentinel rhesus monkey (M-15) serum No. 647495 diluted 1:10	9.4	≤ 10 (10^{-9})	7.5	9.3	7, 8 (10^{-8})	8.8	11 (10^{-8})	n.d.	–	7.5	12 (10^{-6})

[a] Day postinoculation where the endpoint dilution (in parentheses) was reached.
[b] Fluids screened for CPE in Vero cells.
[c] n.d. = Not done.
[d] All cultures or mice were negative at 10^0 and 10^{-1} dilutions.
[e] Zoned, 1/3 negative at 10^0.
[f] Zoned, negative at 10^0.
[g] Pattern of death: 1/8 at 10^0 and 1/7 at 10^{-1} dilutions.
[h] Zoned, 4/4 negative at 10^0.

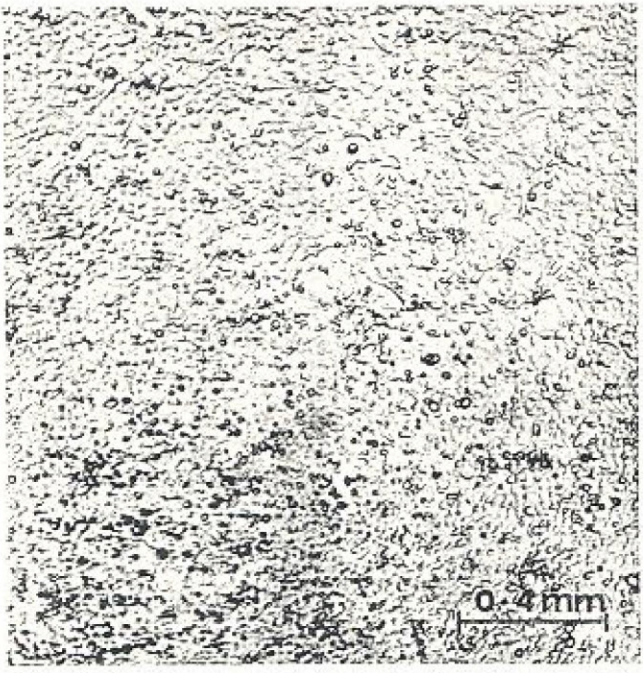

Fig. 1. A. pseudoscutellaris cells; uninfected control.

in the *A. pseudoscutellaris* cells. Small foci of dark granular cell clumps were observed as early as 4 days, progressing rapidly to complete cell destruction involving the whole cell sheet (fig. 1, 2). At this stage almost all the cells had come off the surface of the flask and floated freely in the medium. The endpoint was usually reached in 6–10 days, in one case in 13 days. Extensive CPE was attained even in cultures inoculated with the endpoint dilutions, and the progressive CPE made scoring much easier since it usually involved the whole cell sheet within a few days. The sentinel monkey serum showed the highest titer, 9.4 dex, as determined by CPD_{50} in the *A. pseudoscutellaris* cells. Extensive CPE at the endpoint dilution of 10^{-9} was attained in about 10 days. The mosquito suspensions had titers of 4.9 and 3.9 dex, respectively, and the endpoint dilutions of 10^{-4} and 10^{-3} were reached in 6–10 days. The human liver suspensions gave the lowest titers and the CPD_{50} of both was 1.9 dex. The endpoint dilution of 10^{-2} for 311376 was reached in 7 days and 10^{-1} for 311584 in 9 days in one flask and in 13 days in the other. However, the first-passage mosquito cell culture fluid of strains 311376 and 311584 had CPD_{50} titers of 7.9 and 8.4 dex when titrated in *A. pseudoscutellaris* cells.

Fig. 2. A. pseudoscutellaris cells; 10 days after infection with 10^{-9} dilution of sentinel monkey serum (sample No. 902677) to show extensive cell destruction.

None of the strains produced a CPE in the *A. albopictus* cells, and the infectivity titers (ID_{50}) of fluids from the *A. albopictus* cultures as measured in Vero cells were lower than the CPD_{50} titers in the *A. pseudoscutellaris* cells. Suckling mouse intracerebral titers were lower; the endpoint dilutions were also generally lower than in *A. pseudoscutellaris* cells (in the case of the sentinel monkey serum, 3 dex lower) and incubation periods were as long as 12 days.

Discussion

BUCKLEY [3] obtained multiplication of the Asibi and Couma strains of YF virus in SINGH's *Aedes aegypti* and *A. albopictus* cell lines; in the *A. albopictus* cells both strains produced only transient and sporadically occurring CPE, and she rated these as negative. CORY and YUNKER [11] and YUNKER and CORY [12] obtained clearly defined plaques in *A. albopictus* cells infected with

the 17D strain of YF virus incubated at 37° under an agarose overlay. In our *A. pseudoscutellaris* cells, 17D failed to produce a CPE. Cells infected with the French neurotropic strain of YF developed CPE but not consistently, and the degree of CPE was considerably reduced on passaging the culture medium into fresh *A. pseudoscutellaris* cells.

The usefulness of cultured mosquito cells for primary isolation of arboviruses from naturally infected arthropods or vertebrates depends to a large extent on whether the viruses produce a distinct and reproducible CPE in the cells. Growth of virus in the cells without CPE, appearance of CPE only after a long period of time or production of CPE only after more than one passage seriously reduces the simplicity sought in a cell culture system for primary isolation, particularly if large numbers of specimens are to be screened.

The lower susceptibility and the higher cost of experiments involving mice do not make mouse inoculation the system of choice for primary isolation of YF virus. *A. pseudoscutellaris* cells are easy to maintain and the YF virus in the field samples consistently produced a distinct and progressive CPE which was different from the syncytium formation which other flaviviruses produce in these cells as well as in *A. albopictus* cells. The specificity of this type of CPE produced by YF virus has already been demonstrated in the London laboratory [unpublished data] using a monkey liver strain of YF virus from Trinidad which had been passed once in an *Aotus* monkey. CPE produced by infected serum from this monkey in *Aedes pseudoscutellaris* cells was completely blocked by YF immune serum. Titers of the five YF virus strains in the *A. pseudoscutellaris* cells were of the same order as those in Vero cells on primary isolation. Taking into consideration the titers, the endpoint dilutions and particularly the incubation periods, *A. pseudoscutellaris* cells would appear to be marginally better than Vero cells for primary isolation of YF virus from the Panamanian field samples; it is possible that strains of YF virus from other localities may behave differently in the mosquito cells. Finally, isolation of strains from wild-caught mosquitoes in the mosquito cell line makes it possible to recover and maintain strains of YF without ever passing them in vertebrates or vertebrate cell lines, thus avoiding possible alterations in biological characteristics which may be brought about by cultivation in mammalian systems.

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