Due to low salinity.—According to Brongersma Sanders[1] mass mortality of marine organisms occur whenever there is a great inflow of freshwater due to heavy rains. When the north-east monsoon set in during October–December, freshwater inflow was very high in the Vellar River and due to this low salinity values ranging from 0–15‰ were recorded in the near-shore waters, when several factors coincided—rainfall, sudden influx of freshwater, etc. But this low salinity alone cannot be taken into account for the present mortality, since during previous monsoon periods also the same values were recorded.

Due to cyclonic storm.—From the weather forecast from Madras, in the night of 21st November and 22nd day and again on 6th December, 1972 severe cyclonic storm crossed the Tamil Nadu coast close to Cuddalore. This cyclonic storm combined with spring tide would have caused the large-scale death of *Lingula*.

Severe storms may cause great injury by covering the benthic fauna with layers of mud or sand or conversely benthic invertebrates are plowed from the bottom of the sea and thrown upon the beach[2]. During a severe gale in the Black Sea thousands of decapod crustacean *Upogebia littoralis* Risso which live in burrows were repeatedly cast on shore after storm[3].

The incidence of mass mortality of invertebrates in the Black Sea is of particular interest in view of similar occurrence recorded from other parts of the world. *Lingula* living in the bottom would have uprooted from their burrows by the agitation of water due to cyclonic storm and were cast on the beach. It seems likely therefore in the absence of other factors normally associated with mass mortality[1] that the contributory factor here is cyclonic storm and low salinity.

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INTRACELLULAR AND EXTRACELLULAR COMPLEMENT FIXING ANTIGENS OF JAPANESE ENCEPHALITIS AND CHANDIPURA VIRUSES IN MOSQUITO TISSUE CULTURES

ABSTRACT

Studies were undertaken to determine the intracellular and extracellular distribution of complement fixing (CF) antigens produced in *Aedes albopictus* (ATC-15) and *Aedes w-albus* cultures inoculated with different dosages of Japanese encephalitis (JE) and Chandipura (CHP) viruses.

The results indicated that in JE infected *Aedes albopictus* and *Aedes w-albus* cultures, CF antigens were found both in the extracellular medium and intracellular material whereas, in CHP inoculated cultures, the antigens were retained mainly intracellularly.

Cultures infected with a higher dose of CHP virus showed less complement fixing antigens indicating the tendency to develop prozone phenomenon. Such prozone phenomenon was not observed with JE infected cultures.

MOSQUITO tissue culture has been extensively used for studying the growth of arboviruses[1]. Pavri and Ghosh[2] detected complement fixing (CF) antigens in *Aedes albopictus* (ATC-15) cell cultures infected with dengue viruses; thus providing a simple method of identification of the type of virus involved. In their study, frozen and thawed culture fluids containing both intra- and extracellular viral antigens were employed in CF test and no attempt was made to determine intra/extracellular distribution of viral antigens separately.

This communication presents the preliminary results of distribution of CF antigens in *Aedes albopictus* and *Aedes w-albus* cultures inoculated with different dosages of Japanese encephalitis (JE) and Chandipura (CHP) viruses. Both JE and CHP viruses have been found to grow in *Aedes albopictus*[3,4] and *Aedes w-albus*[5] cultures.

The methods of preparation of cultures of *Aedes albopictus* (passage level 47) and *Aedes w-albus* (passage level 109) were essentially the same as described earlier[3]. Both JE (VRC No. P 20778; mouse passage level 16; titre 6·80 log mouse $LD_{50}/0·03$ ml for adult mice) and CHP (VRC No. 653514 mouse passage level 22; titre 7·75 log mouse $LD_{50}/0·03$ ml for adult mice) viruses were obtained from brains of infected baby mice as 20% suspensions in phosphate saline (pH 7·2) containing 0·75% bovalbumin and stored at − 50° C in sealed ampoules.

The cultures were inoculated with tenfold dilutions of JE and CHP virus stocks according to the method[3] described previously. The extracellular culture fluids were removed on the 4th PI day in

case of JE virus and on the 6th PI day in the case of CHP virus as these days were found to be optimum for antigen production by the respective viruses (unpublished data). The intracellular materials were obtained by washing the cell sheet

employing respective immune sera according to the method described by Pavri and Shaikh[6].

CF antigens in *Aedes albopictus* (ATC-15) and *Aedes w-albus* cultures infected with JE virus were present both intra- and extracellularly (Table I).

TABLE I

Distribution of JE and CHP complement fixing antigens intra- and extracellularly in
Aedes albopictus *and* Aedes w-albus *cultures*

Virus	Cultures	Dosage of the virus inoculated†							
		7·75		6·75		5·75		4·75	
		EC	IC	EC	IC	EC	IC	EC	IC
JE	*Aedes albopictus*	ND	ND	$\frac{128}{8}$	$\frac{256}{8}$	$\frac{128}{8}$	$\frac{256}{\geqslant 16}$	$\frac{128}{\geqslant 16}$	$\frac{256}{\geqslant 16}$
	Aedes w-albus	ND	ND	$\frac{256}{4}$	$\frac{256}{16}$	$\frac{256}{\geqslant 16}$	$\frac{256}{\geqslant 16}$	$\frac{256}{\geqslant 16}$	$\frac{256}{\geqslant 16}$
CHP	*Aedes albopictus**	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{8}{2}$	$\frac{<8}{0}$	$\frac{64}{4}$
	Aedes w-albus	$\frac{8}{UD}$	$\frac{16}{UD}$	$\frac{8}{UD}$	$\frac{16}{UD}$	$\frac{8}{UD}$	$\frac{128}{2}$	$\frac{128}{UD}$	$\frac{128}{4}$

Virus	Cultures	Dosage of the virus inoculated†							
		3·75		2·75		1·75		0·75	
		EC	IC	EC	IC	EC	IC	EC	IC
JE	*Aedes albopictus*	$\frac{64}{\geqslant 16}$	$\frac{256}{\geqslant 16}$	$\frac{128}{8}$	$\frac{256}{\geqslant 16}$	$\frac{256}{8}$	$\frac{256}{\geqslant 16}$	$\frac{128}{8}$	$\frac{256}{8}$
	Aedes w-albus	$\frac{256}{8}$	$\frac{256}{\geqslant 16}$	$\frac{128}{UD}$	$\frac{16}{8}$	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{<8}{0}$	$\frac{<8}{0}$
CHP	*Aedes albopictus**	$\frac{<8}{0}$	$\frac{128}{8}$	$\frac{<8}{0}$	$\frac{128}{8}$	$\frac{<8}{0}$	$\frac{128}{8}$	ND	ND
	Aedes w-albus	$\frac{128}{UD}$	$\frac{128}{8}$	$\frac{128}{UD}$	$\frac{128}{8}$	$\frac{64}{UD}$	$\frac{128}{8}$	ND	ND

* Undiluted extracellular fluid in some samples showed low level of CF antigens.
† Dosage calculation vary ± 0·05.
Numerator indicates highest titre of antisera.　Denominator indicates highest titre of antigen.
　UD = Undiluted;　　EC = Extracellular;　IC = Intracellular;　ND = Not done.

thrice with balanced salt solution and disrupting the cells by repeated freezing and thawing with the same quantity of fresh medium. It was then centrifuged at 2,000 g for 15 minutes to discard the cell debris. Both the extracellular culture medium and intracellular material were used as CF antigens

In *Aedes albopictus* culture CF antigens were detected in all the cultures inoculated with virus dosages varying from 6·80 log mouse LD_{50} to 0·80 log mouse LD_{50}. In *Aedes w-albus* culture, however, no antigen could be detected in the dosages of 1·80 and 0·80 log mouse LD_{50}. This

could be due to the fact that *Aedes albopictus* culture was more sensitive to JE virus than *Aedes w-albus* culture as evidenced from the comparative yield of infectious virus from the respective cultures (unpublished data). In general, the intracellular material showed slightly higher concentration of CF antigen than the extracellular medium.

With CHP virus the CF antigens remained mostly intracellular both in *Aedes albopictus* and *Aedes w-albus* cultures (on the 6th PI day). None of the extracellular culture media of CHP infected *Aedes albopictus* cultures reacted in CF test whereas extracellular media of CHP infected *Aedes w-albus* cultures showed presence of CF antigens only in the undiluted samples. However, the intracellular material (CHP infected *Aedes w-albus* cultures) contained higher concentration of CHP complement fixing antigens than that present in the extracellular medium.

It was also found that a higher dose (7·75, 6·75 and 5·75 log mouse LD_{50}) of CHP virus both in *Aedes albopictus* and *Aedes w-albus* cultures resulted in lower yield of CF antigens as compared to the inoculum containing lower concentrations of virus (1·75 to 4·75 log mouse LD_{50}). This type of prozone phenomenon has been also observed with regards to infectivity of CHP virus in vero, *Aedes albopictus* and *Aedes w-albus* cultures (VRC unpublished data 1971 and 1972). However, this was not observed in case of JE infected cultures.

The results thus indicate that in JE infected *Aedes albopictus* and *Aedes w-albus* cultures, CF antigens were distributed both extra- and intracellularly whereas with CHP virus the CF antigens were located mainly intracellularly.

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CHROMOSOME STUDIES IN THE LIVER FLUKE, *FASCIOLA GIGANTICA* COBBOLD, 1856, FROM ANDHRA PRADESH

The genus *Fasciola* claimed to consist of three[1] to six species[2] enjoys a wide range of mammalian hosts including the humans. The variation in the elongated shape of *F. hepatica* from ox in contrast to the oval of that from sheep is considered to be due to adaptive factors acting in the final host. Experimental infection of *F. gigantica* in rabbits[3] indicates that apart from genetic factors, physiology and the environment of the host play a major role on the parasite morphology. Apparently a thin line of distinction exists between *F. hepatica* smaller than *F. gigantica*[4] with the consequential changes in the orientation of the reproductive organs and slight variations in life-histories. Comparative cytology may throw some light in this direction. However, the trematode cytology has been considered to be difficult in view of the application of elaborate mammalian chromosome schedules[5] and heavy condensation of meiotic chromosomes precluding clear morphology[6]. The cytology of digenetic trematodes is still a matter of speculation and divergence of opinion in spite of the chromosome numbers being reported in several of them by Britt[7] and Walton[8]. The diploid chromosome number of *F. hepatica* has been variously claimed as eight[9], twelve[10–12] or as eighteen or twenty[13–15]. The haploid number has been reported to vary from six[11·12] to nine and ten[14·15] bivalents. There is also a lack of knowledge on the karyotype of this form. While Srivastava and Jha[16] have described the chromosome number of *F. gigantica* from the liver of buffalo as sixteen with eight bivalents, based on observations from only spermatogenesis, our studies, however, indicate the same to be twenty with a haploid count of ten bivalents. The chromosome number and the karyotyping from meiotic as well as mitotic cells of *F. gigantica* have been reported here.

The adult parasites from the bile ducts of sheep and the larval forms from the infected snails, *Lymnaea luteola*, were collected from Banswada in the Nizamsagar Irrigation area in Andhra Pradesh, where the incidence of Fascioliasis is known to be high. The technique of handling the adult parasite material has been described elsewhere[17]. The first report of obtaining mitotic chromosome complements from larval stages of trematodes was by Cary[18] and later followed by Short and Menzel[19] and Ching Tsong Lo[20]. As the older rediae lack the germinal material the