temperatures due to lower rates of viral multiplication (Danielova, 1975; Hardy et al, 1983), can be extremely important to the epidemiology of the arbovirus. This is because the period the mosquito must survive after an infective meal until transmission occurs will determine if the mosquito can still be a competent vector. Studies have also indicated that incubation temperatures affect dissemination and infection barriers within the mosquito and very often gives rise to rather inconsistent results (Hardy et al, 1983; Kramer et al, 1983). Virus multiplication varies within different species of mosquito and the same virus often has different EI periods in different species of mosquito even if the mosquitoes are held at the same temperature (Chamberlain et al, 1955; 1959). It is therefore clear that each virus has its own unique relationship with a mosquito vector, and the effects of temperature on vector competence should be studied for each virus and vector. Many such studies have been done and the discussion of these studies is divided into three main groups according to the endemic climatic distribution of the virus concerned.

D.1. Viruses occurring in the cooler regions of the world.

a) Snowshoe hare virus (Bunyaviridae).

Snowshoe hare (SSH) virus, a subtype of CE virus has been isolated from several species of Aedes mosquitoes, particularly Ae. canadensis (Mclean et al, 1971) in North America as far north as Alaska. These mosquitoes are active and feed on humans in the field at temperatures of 10°C and lower (Mclean et al, 1974). Higher temperatures of 13°C and 26°C did not change the EI period for SSH virus in orally infected Ae. aegypti mosquitoes and virus was transmitted after 28 days at both these temperatures (Mclean et
al, 1974). However, *Ae. aegypti* is neither a natural vector of SSH virus nor an efficient laboratory vector. In spite of this, studies with *Ae. aegypti* do indicate that SSH virus multiplies and can be transmitted at low temperatures.

b) Whataroa virus (Togaviridae).

Whataroa virus (Alphavirus) is endemic to New Zealand which experiences diurnal average monthly summer temperatures of 16°C, with maximum temperatures of 21-22°C, and diurnal average monthly winter temperatures of 6°C. Main vectors of WHA virus are the mosquitoes *Culex pervigilans* and *Culiseta tonnori* (Miles et al, 1973). Since the workers were not able to keep these species alive sufficiently long enough in the laboratory, the multiplication of WHA virus was studied in another mosquito, *Aedes australis*, which also survives best at cool temperatures with maximum survival at 20°C. Most *Ae. australis* became infected and transmitted virus 17 days after imbibing the infective blood meal at 20°C indicating that WHA virus is adapted to replicate in mosquitoes at relatively low EI temperatures.

D.2. Viruses from warm regions (tropical to sub-tropical).

Most studies on the effects of temperature on vector competence with viruses endemic to the tropics have been conducted with the following flaviviruses: YF virus; DEN virus and JBE virus. The results obtained from a few studies on these viruses are summarized in Table 1. These viruses are clearly strongly influenced by EI temperature, with infection and transmission rates rapidly declining at temperatures below 30°C. Reduction in rates of replication of these viruses within the mosquito due to lower temperatures not only
reduces infection and transmission rates, but also lengthens the EI period. At temperatures less than 15°C virus multiplication is so low that the mosquitoes no longer seem to be infected, particularly with YF and DEN viruses. Nevertheless, virus infection at these lower temperatures still persists as rapid virus replication occurs when mosquitoes held at low temperatures are transferred to higher temperatures (Davis, 1932; Bates and Roca-Garcia, 1945; 1946; LaMotte, 1963). JBE virus seems to have an unusually broad temperature tolerance, as it is transmitted by different mosquito species in different climatic regions (Huang, 1957). It is probably due to this greater temperature tolerance of JBE virus that periodic epidemics of the virus have occurred during summer periods in temperate climates in Asia (Schichijo et al, 1972; Hayashi et al, 1976), although the virus is endemic to tropical Asia (Bhattacharya et al, 1966). The main vector of JBE virus in tropical Asia is Cx. vishnui (Bhattacharya et al, 1986), while in temperate areas it is Cx. tritaeniornynchus (Hayashi et al, 1976).

D.3.
Viruses from temperate areas.

a) Western equine encephalitis virus (Togaviridae)

Strains of Cx. tarsalis resistant or susceptible to oral infection with WEE virus have been selected and female F1 progeny from crosses between these strains exhibit mesenteronal or salivary gland infection barriers (Kramer et al, 1983). The F1 females were orally infected with WEE virus and transmission and infection rates determined at different temperatures. The mosquito was found to be a less competent vector after 2-3 weeks extrinsic incubation at 32°C than after incubation at 18°C or 25°C (Kramer et al, 1983). These authors thus reported they had shown a case
Table 1.
Summary of results obtained from temperature vector competence studies on viruses occurring in the tropics.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Period (days)</th>
<th>Infection</th>
<th>Transmission</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarbash, 1970</td>
<td>0-60 (20-35)</td>
<td>0/10 (14-23)</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Huang, 1975</td>
<td>8</td>
<td>0/6 (10-18)</td>
<td>0/20 (2-10)</td>
<td>80-85</td>
</tr>
<tr>
<td>1974.5:19746</td>
<td>0/10</td>
<td>0/6 (10-18)</td>
<td>0/20 (2-10)</td>
<td>80-85</td>
</tr>
<tr>
<td>1974.5:19746</td>
<td>0/10</td>
<td>0/6 (10-18)</td>
<td>0/20 (2-10)</td>
<td>80-85</td>
</tr>
<tr>
<td>Barcis, 1974</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Davis, 1973</td>
<td>18</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>4-5</td>
<td>6</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Martin et al., 1975</td>
<td>32</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Martin et al., 1974</td>
<td>12</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Martin et al., 1974</td>
<td>12</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Watts et al., 1987</td>
<td>7</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
<tr>
<td>Deng, 1974</td>
<td>2</td>
<td>0/0</td>
<td>0/0</td>
<td>70-80</td>
</tr>
</tbody>
</table>

VIRUS: Culex Species of Route of Infection Temperature Incubation Rate Extrinsic Reference
where an inverse relationship existed between the vector competence of a mosquito for an arbovirus and the temperature of EI period within the range 18 to 32°C. They believed the phenomenon was related to the mosquito's ability to limit multiplication and/or dissemination of the virus which was in turn controlled by various dissemination, transmission and infection barriers within the mosquito body. The vector competence of wild strains of *C. tarsalis* orally infected with WEE virus responds similarly to incubation temperature although perhaps not as markedly as that of the F1 progeny from crosses between WR and WS strains of *C. tarsalis* (Kramer et al, 1983). Maximum transmission rates of WEE virus by *C. tarsalis* is therefore only obtained at certain temperatures and this could account for the endemic temperate distribution of WEE virus. Even so it cannot be stated what these optimal temperatures are since there is also geographical genetic variation in the mosquito with respect to vector competence. For example, certain strains of *C. tarsalis* are adapted to cooler temperatures and therefore occur further north in the colder Saskatchewan region of North America which experiences diurnal average summer temperatures between 21-24°C (Hayles et al, 1972). Vector competence studies on these mosquitoes have shown that they effectively transmit a local strain of WEE virus at both 21°C and 24°C, 4 to 44 days after an infective blood meal.

b) St. Louis encephalitis virus (Flaviviridae)

Temperature vector competence studies have been conducted on *C. quinquefasciatus* (Say) mosquitoes infected with SLE virus (Hurlbrut, 1973) and as with WEE virus, the maximum transmission of SLE virus was only obtained within a certain temperature range, with maximum transmission reached at 30°C. This study was perhaps unique in that Hurlbrut was able to
demonstrate that EI period and blood digestion time were approximately linearly related to temperature between 20-30°C. Furthermore, survival of this species of mosquito to completion of virus incubation was also shown to be optimal at a mean incubation temperature of 30°C.

If transmission of SLE virus by *Cx. quinquefasciatus* is compared to transmission of WEE virus by *Cx. tarsalis*, it seems that *Cx. quinquefasciatus* is better adapted to transmit SLE at higher temperatures than *Cx. tarsalis* is able to transmit WEE virus. This could explain why environmental temperatures usually need to be slightly higher for epidemics of SLE virus than for WEE virus. Evidence has in fact accumulated suggesting activity of WEE and SLE viruses in Kern County, California, usually follow half month periods of mean daily temperatures exceeding 27°C and 31°C respectively (Reeves et al., 1964; Kramer et al., 1983). Furthermore, SLE virus activity follows unusually warm spring temperatures while high WEE viral activity most frequently occurs after abnormally cool spring temperatures. Associated with this, some workers even go so far as to state that epidemics of WEE virus usually occur at or above the 25°C June isotherm whereas those of SLE virus usually occur in areas below this isotherm (Hess et al., 1963).

c) eastern equine encephalitis virus (Togaviridae)

The mosquito *Ae. triseriatus* was shown to transmit EEE virus mechanically for periods of up to 3 days after an infective feed at temperatures of 21°C (Chamberlain and Sudia, 1955). Mechanical transmission was however completely eliminated at 32°C and Chamberlain and Sudia (1955) considered that the more rapid inactivation of virus and greater cleansing of mouthparts by the mosquito at higher temperatures accounted for this. A similar trend in reduction of mechanical transmission at higher temperatures was
also shown with JBE virus and \textit{Cx. tritaeniorhynchus} mosquitoes (Hayashi et al., 1976). In \textit{Ae. triseriatus}, however, an increase in temperature from 21°C to 32°C had no effect on infection rates or titers of EEE virus reached in individual mosquitoes, although higher transmission rates and shorter EI periods were observed at the higher temperature. The subjection of mosquitoes to temperatures of 32°C for 4 hours and 21°C for 20 hours affected vector competence, causing intermediate transmission rates and EI periods as compared to the mosquitoes held at a constant 32°C and 21°C.

d) Rift Valley fever virus (Bunyaviridae)

Incubation temperatures were shown to influence the vector competence of \textit{Cx. pipiens} and \textit{Ae. taeniorynchus} with RVF virus (Turrell et al., 1985). The virus replicated more rapidly at higher temperatures in both species after oral ingestion of virus, so that there was an inverse relationship between EI period and incubation temperature. Infection rates in the 2 species, however, differed in response to temperature, with rates in \textit{Cx. pipiens} significantly higher at 33°C (95%) than at 13°C (38%), whereas they remained unchanged in \textit{Ae. taeniorynchus}. Of further significance is that the low infection rate obtained at 13°C (38%) in \textit{Cx. pipiens} increased in mosquitoes that were transferred from 13°C to 28°C. It appears that at low temperatures the virus is not lost, but rather falls to undetectable levels or enters some sort of latent infectious phase. This has been shown to occur with several other arboviruses already mentioned in Section 1.1.2.D.2.

e) Tahyna virus (Bunyaviridae)

TAH virus is endemic to Central Europe where it is transmitted by \textit{Ae. vexans}. In an effort to study the
Effect of temperature on the virus. Malkova and Marhoul (1976) inoculated it into an *Ae. albopictus* cell culture line to observe viral multiplication at 28°C and 20°C. These temperatures correspond to the average temperatures experienced by the mosquito vector during its summer activities in Central Europe. Titres were slightly lower at 20°C. Hence it seems that TAH virus is not markedly affected by temperature, but that its distribution is restricted to temperate regions where the natural vector *Ae. vexans* occurs.

1.2. Epidemiology of WN and SIN viruses in South Africa.

Two arthropod-borne viruses, namely WN and Sindbis (SIN) viruses are enzootic in the highveld region of South Africa which experiences a temperate climate. These viruses have also been isolated in many other regions of the world including parts of Africa, Asia and Europe (Smithburn et al., 1940; Woodall et al., 1961; Weinbren, 1955; Taylor et al., 1956; Goldblum et al., 1958; Shah et al., 1960; Pavri and Singh, 1965; Filipe, 1972; Hayes et al., 1982; McIntosh, 1986) and SIN virus only from Australia (Doherty et al., 1969). Both WN and SIN viruses are epidemiologically similar in that they are avian-borne viruses with the mosquito *Culex univittatus* as the main vector. Serological surveys done on birds in Egypt (Work et al., 1953; 1955; Taylor et al., 1955), South Africa (Kokernot et al., 1956; McIntosh, 1986) and Asia (Shah et al., 1960) have shown that several species of birds play a major role in the epidemiology of these viruses. Many of these birds migrate between northern and southern Africa and Asia and could therefore serve as important seasonal sources of the viruses resulting in the widespread distribution of these viruses. Many isolations of these viruses, particularly WN virus have been made from *Culex* mosquitoes in Egypt (Work et al., 1953), Pakistan (Akhter et al., 1982), India (Pavri et al., 1965), Arabia (Wills et
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In South Africa most isolations of both WN and SIN viruses have been made from the mosquito _Cx.univittatus_ Theobald (Mcintosh et al, 1986; Jupp and McIntosh, 1967; Jupp et al, 1986) from the inland plateau region. _Cx.univittatus_ is mainly ornithophilic, but will feed on man and probably more so when abundant after heavy rainfall. The mosquito plays a major role in maintaining the avian-borne cycles of the viruses due to its ornithophilic feeding preferences, but on occasions bites and could infect humans who then become secondarily involved. These viruses both cause a mild illness in man, an account of which is given by McIntosh (1986).

In the past, isolations of both WN and SIN viruses have also been made from 2 other species of mosquitoes which also commonly occur in the inland plateau region. These are _Cx.pipiens_ L. and _Cx.theileri_ Theobald (McIntosh et al, 1978). Isolations from these two species of mosquitoes have been far fewer than from _Cx.univittatus_ which may be because these two species of mosquitoes feed preferentially on birds that could be relatively resistant to infections with the viruses (Jupp et al, 1972; Jupp, 1976).

Although all 3 species are highly susceptible to both viruses when orally infected by feeding on viraemic chicks, only _Cx.univittatus_ is capable of transmitting the virus efficiently (Jupp, 1976). Serological surveys on the inland plateau and in the Natal coastal lowlands have shown that human immune rates to both WN and SIN viruses are much higher in the inland plateau region (Jupp et al, 1986). _Cx.univittatus_ is mainly restricted to the higher inland plateau and is replaced by a similar but more anthropophilic species of mosquito called _Culex neavei_ (Theobald) in the lower coastal regions. _Culex neavei_ is, however, less susceptible to WN and SIN viruses than _Cx.univittatus_ with 50% infection thresholds 2 logs higher in the case of WN virus and 1 log in the case of SIN virus. Titres of
both viruses often develop below the infectivity thresholds of *Cx. neavei* in birds, which could account for the poor vector capability and hence the lower human immune rates and fewer incidences of clinical infections in the Natal coastal lowlands (Jupp et al., 1986).

A few cases of WN and SIN viruses occur every summer particularly in the moist regions of the Transvaal and Orange Free State (Jupp et al., 1986). However, in early 1974 the largest epidemic of both these viruses ever recorded in South Africa occurred in the usually arid Karoo and Northern Cape Province with thousands of human infections estimated to have occurred (Mcintosh et al., 1976). That summer, the region received exceptionally high rainfall (Fig. 2) which resulted in large populations of mosquitoes. This together with the custom of many of the local people of sleeping outside because of the summer heat is thought to have led to the higher number of cases. A higher than usual number of human cases of SIN virus and to a lesser extent WN virus also occurred in the Witwatersrand-Pretoria region from December 1983 to April 1984 (Jupp et al., 1986). The above two incidents are unusual for viruses that are normally enzootic with sporadic human cases. During these epidemics *Cx. univittatus* infection rates were markedly higher than usual which confirmed the important role that this species plays both in maintenance of the virus and in infecting humans during epidemics. Jupp et al. (1986) reported that the higher than normal temperatures recorded during the summer of 1983 and 1984 (Fig. 3a) and higher than usual early summer rainfall (Fig. 3b), might have favoured viral infections in the mosquito and subsequently enhanced transmission of the viruses in the Witwatersrand-Pretoria region. Second, preliminary studies by Jupp (1974) on the transmission of WN virus at different temperatures demonstrated that a lowered temperature reduced the viral transmission rate 17 days after the infective feed, but
Total monthly rainfall figures in mm during the summer months from 1970-1980 in Upington.
did not reduce the infection rate. Jupp also mentioned that in the plateau region, particularly the Highveld, under normal summer conditions, daily temperatures fluctuate markedly which is typical of temperate climates. Therefore to get a better overall understanding of the dynamics of viral transmission by Cx. univittatus it seemed important to investigate the effects of fluctuating temperatures on vector competence, and to compare this with similar studies carried out at constant temperatures.

The present study further investigated the effect of temperature on the vector competence of Cx. univittatus for WN and SIN viruses respectively, "vector competence" being the susceptibility to virus infection and ability to transmit virus. Groups of infected mosquitoes were held at constant temperatures of 14, 18, 26 and 30°C and another group at the fluctuating ambient temperature outdoors in the late summer in Johannesburg. Limited studies were also conducted on the longevity of uninfected mosquitoes at different temperatures, and a comparison was made between the longevity of uninfected and SIN and WN virus-infected mosquitoes held at 28°C. Combined with knowledge of the geographical location, temperature and rainfall figures of past epidemics of both viruses, the results of these temperature vector competence and longevity studies contribute towards a better understanding of the factors that influence outbreaks of these viruses in South Africa. Replication and the distribution of each virus in Cx. univittatus has not yet been studied, and hence this was investigated by fluorescent antibody tests on the various organs of the mosquito.
Fig. 3

a) Average maximum and minimum temperatures in °C experienced during the summer months in Johannesburg from 1976-1986.

b) Total monthly rainfall in mm in Johannesburg during the summer months of 1976-1986.
CHAPTER 2

MATERIALS AND METHODS.

2.1. Mosquito colony and rearing.

Methods for rearing *Cx. univittatus* mosquitoes were essentially the same as those described previously by Jupp (1967). Mosquitoes were collected in the summer of 1985 near the Klip river in Johannesburg using portable baited traps which were based on the lard-can bait trap designed by Bellamy and Reeves (1952). Bait employed was a single domestic pigeon (*Columba livia*) which was restrained within a nylon stocking. These traps were suspended from trees about 1-2 meters above the ground and left overnight. The following morning the traps were brought back to the laboratory, where the engorged *Cx. univittatus* mosquitoes were removed and placed in cages measuring 35x35x35cm in an insectary. Conditions in the insectary were maintained at 26±2°C, 75-80% relative humidity (RH) and a photoperiod of 11hrs complete darkness and 11hrs of maximum light with a 1hr crepuscular period in the late afternoon and early morning. Eggs were initially obtained from individual gravid females by placing them in 2.5cm diameter glass tubes half filled with grass infusion. This method was only used for a few generations, and afterward eggs were just as efficiently obtained by placing black dishes filled with grass infusion into the cages. The mosquitoes deposited egg rafts on the surface of the water overnight, which were carefully removed the next morning and placed in beakers of distilled water for hatching. The larvae were placed in enamel pans (27x37cm) in water with a few pieces of dried Kikuyu grass and given a daily diet of Brewers yeast (500mg potency manufactured by Vita force) and
"Nestum" (mixed baby cereal manufactured by Nestlé), and airated. The newly emerged progeny of field mosquitoes were subsequently added to cages containing recently emerged adults from an older established laboratory colony (F8) of Cx. univittatus that also originated from individuals collected in Johannesburg. This mixed colony of wild and colonized mosquitoes provided the initial material for the vector competence studies. Before any experiment, large numbers of young adult mosquitoes were reared using the techniques described above, of which half the adults reared were used for vector competence studies and the other half for maintaining the colony and providing the next generation of mosquitoes for the next experiment. All adult mosquitoes were maintained on a diet of a 4% sucrose solution. In the summer of 1986 more field mosquitoes were collected and their offspring also added to the colony. The initial establishment of a colony only incorporates a portion of the field population’s genetic variability, and fluctuations in colony size during colonization sometimes reaching very low levels could also add to genetic change due to stochastic processes of genetic drift (Lorenz et al., 1984). For this reason and for the reasons discussed earlier in chapter 1, viz., colonization resulting in a change in vector competences of mosquitoes, the colony was kept as large as possible by rearing offspring from every egg raft obtained, and wild mosquitoes were added to the colony during the summer months. Such mosquitoes were used throughout the project.

2.2. Virus.

The H442 strain of WN virus used was originally isolated from a human being at Ndumu, South Africa, in 1959 (Kokernot and McIntosh, 1959).
The AR86 strain of SIN virus used was originally isolated in 1954 from a pool of mixed Culex mosquites collected in Springs, east of Johannesburg (Weinbren et al., 1956). Both viruses had been passaged five times in mice and four times in Vero cells.

2.3 Oral infection of Culex univittatus.

2.3.1 Membrane feeding.

Two different artificial feeding techniques and four different membranes were evaluated to establish a method whereby a large number of mosquitoes could be fed efficiently on defibrinated blood. It was hoped that such an artificial feeding method could be used to infect the mosquitoes by feeding them on blood-virus mixtures, permitting the control of the viral titre in the mixtures. One of the artificial techniques used was developed by Davis et al. (1983). Here the blood was citrated, kept at 32°C and constantly stirred. The other feeding technique was that of Jupp and McIntosh (1970) where defibrinated blood was maintained at 32°C without stirring. The four membranes tested were the Badruche membrane, stretched Parafilm (American Can Company, Greenwich, U.S.A.), freshly harvested skin from 2 week-old chickens and a silicon membrane (Davis et al., 1983). Each of these membranes was evaluated with both feeding techniques.

2.3.2 Viraemic chicks.

The experiments required large numbers of mosquitoes infected with a high dose of virus. To meet this need the method previously employed by Jupp (1976) was adopted. Maximal titres of SIN virus were obtained by inoculating 1-day-old chicks intramuscularly, and exposing them to mosquitoes 24 hours later. On the other hand, maximal titres of WN virus were obtained by inoculating 2-day-old chicks and exposing them to mosquitoes 36 hours later. Just prior to infecting the
mosquitoes, the concentration of virus in the blood of each chick was determined. For this, 0.1ml of blood was removed from the wing vein, diluted with 0.9ml of Leibovitz's medium (Gibco Europe Limited, Paisley, Scotland) to which streptomycin, neomycin, penicillin, geromycin and foetal calf serum were added and 10 fold serial dilutions then inoculated onto monolayers of Vero cells. Vero cells respond to both WN and SIN virus infections by visually displaying cytopathic effect. Plaques form after 2 days in the case of SIN virus and 4 days with WN virus. The 50% cytopathic dose (CPD\(50\)) endpoints were calculated by the method of Reed and Muench(1938). Viral titres were expressed as Log\(10\) CPD\(50\)/ml of chick blood. After the chicks had been bled they were restrained inside nylon stockings and left overnight in cages containing 1-week-old mosquitoes. The mosquitoes were housed in an insectary at 26°C and a RH of 75-80% where all of the infective feeds were given. The mosquitoes were starved for 24 hours preceding each infective meal. The following morning the chicks were taken out of the cages and all fully engorged mosquitoes were placed into other cages to be held at the various incubation temperatures.

2.4. **Temperature conditions.**

For vector competence and longevity studies at 26°C, the infected mosquitoes were held in the insectary. However, only the insectary had a constant relative humidity under electronic control and therefore at all the other temperatures the mosquitoes were kept at high humidity, (see section 2.7), by placing a large pad of cotton-wool saturated with water on top of the cages and then covering the whole cage with plastic to retain the moisture. Incubation cabinets (14°C and 30°C) and a temperature-controlled room (18°C) provided the different temperatures required. The lighting regime consisted of 12 hours light and 12
hours darkness. For exposure of mosquitoes to temperature conditions outdoors, a mosquito proof "walk-in" cage measuring 2x2x2 metres was constructed and positioned outside the laboratory away from direct sunlight. Mosquitoes within smaller 35x35x35 cm cages provided with moist pads of cotton wool and covered with plastic, were placed on a table in the middle of the walk-in cage. The mosquitoes were thereby exposed to fluctuating ambient temperature while still held at a high RH within the plastic, and were prevented from escaping by 2 barriers. Thermohygrometers were placed with the mosquitoes at the various temperatures to record any fluctuations in temperature and RH.

2.5. Transmission.

2.5.1. Definitions of infection and transmission rates.

The infection rate (IR) is the proportion of mosquitoes feeding on the infective blood-meal which become infected. The transmission rate (TR) is the proportion of infected mosquitoes transmitting virus. Both are expressed as percentages.

2.5.2. Determination of method for transmission tests.

The method used at present at the National Institute for Virology for transmission studies with WN and SIN viruses and *Cx. univittatus* mosquitoes involves feeding individual infected mosquitoes on susceptible laboratory animals, for example, either individual chicks or individual hamsters. Golden Syrian hamsters, the more convenient animals, respond to SIN virus infections by developing high antibody levels that can be demonstrated after 21 days by the haemagglutination inhibition test (Casals and Brown, 1954). West Nile virus on the other hand is lethal, the hamsters dying 7-10 days after the
Bits of an infected mosquito. Livers are removed from the dead hamsters and tested for virus in infant mice. Although hamsters serve as good laboratory animals for transmission studies with these viruses, very low feeding rates are obtained with ornithophilic Cx. univittatus mosquitoes. As transmission tests needed to be done at regular intervals in the present study the hamster method would have been both time consuming, cumbersome and costly. Hence two other in vitro transmission methods, "droplet and capillary", previously utilized for other viruses, were compared to the transmission rates obtained using hamsters for both WN and SIN viruses. The droplet method developed by Gubler and Rosen (1976) had been found as sensitive as engorgement upon suckling mice for detection of YF virus transmission by Ae. aegypti (Beaty and Aitken, 1979) and for transmission of DEN virus by Ae. albopictus. (Gubler and Rosen, 1976). Using this method, each infected mosquito is placed in a tube covered with fine-mesh nylon material, so that it can feed from a droplet of fluid placed on the material (Fig. 4). While feeding, the insect secretes saliva and virus into the droplet and the unconsumed part of the droplet is then tested for virus to determine whether transmission has occurred. The "capillary" method developed by Aitken (1977) was used successfully for demonstrating transmission of YF virus by Ae. aegypti mosquitoes (Beaty and Aitken, 1979). In this technique a capillary tube is drawn to a fine point narrow enough to fit snugly over the proboscis of a mosquito. The tube is filled with a predetermined amount of feeding suspension. A mosquito is anaesthetized with carbon dioxide and the first two pairs of legs removed to immobilize the insect. The base of the wings are gripped with forceps and the proboscis of the mosquito forced into the narrow opening of the prefilled capillary tube. The tube with the mosquito dangling from it (Fig. 5) is suspended from a polystyrene rack until the insect has fed to
repletion. As with the droplet method, the mosquito secretes saliva and virus into the suspension which can then be tested for presence of virus to demonstrate transmission. In the present study, high feeding rates were obtained when a feeding suspension of equal parts of 10% sugar, foetal calf serum and washed goose erythrocytes was used in the droplet method, and a suspension of equal parts of 10% sugar and foetal calf serum was used in the capillary method. In the droplet method, 25μl of the above solution was placed on the nylon material and after the mosquito had fed the remainder of the drop was diluted 10-fold in 250μl of Leibowitz's medium before inoculation onto Vero cells. In the case of the capillary method, only 4μl of feeding solution could be drawn up into the tube and after the mosquito had fed, only 2.5μl of fluid was left. Therefore the capillary droplet had to be diluted 100-fold with Leibowitz's medium (250μl) before inoculation onto Vero cells. If the Vero cells developed cytopathic effect (CPE) due to the presence of virus in the suspensions then successful transmission had occurred. Transmission rates were determined for Cx. univittatus with WN and SIN viruses respectively by each of the three methods described above to allow a comparison between them. In these experiments infection rates were determined by intracerebral inoculation of infant baby mice with 0.02ml of supernatant prepared from individually homogenized mosquitoes. The mice died 3 days after inoculation if the mosquito was infected with SIN virus and 6 days if the mosquito was infected with WN virus. The titre of infecting chicks, transmission and infection rates and P values calculated from chi square tests applied to the transmission rates are shown in table 3.
Fig. 4.
Droplet method: mosquito feeding (life size).

Fig. 5.
Capillary method: mosquito feeding (twice life size).