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An outbreak of type 2 dengue fever in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse)

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Between December 1976 and September 1977 the Seychelles group of islands in the Indian Ocean was struck by an extensive epidemic of dengue fever. The peak of the epidemic was in the last week of February. Type 2 dengue virus was isolated from patients and mosquitos. Aedes albopictus *was the sole vector. The clinical picture was that of classical dengue. Haemorrhagic fever and the shock syndrome were not observed.*

Absenteeism from schools and offices, anamnestic questioning, and prevalence of antibodies in sera collected after the epidemic was over, indicated that approximately 75% of the population had been infected. Serological evidence was obtained of an epidemic of dengue in the islands more than 40 years earlier. This was confirmed by archival records.

Since 1970, a series of outbreaks of dengue fever has been observed in continental South and Central America and in islands of the Caribbean and South Pacific. Several of the countries and islands in these regions, where epidemics of dengue had not occurred for decades, are now experiencing yearly outbreaks. In addition to the endemic strains, dengue virus types never before recorded in certain areas are causing epidemics, e.g., type 1 has appeared in the western hemisphere (*1*, *2*) and type 4 in the South Pacific (*3*).

The virus has also been reported from islands in the Indian Ocean. Between December 1976 and September 1977, an epidemic of dengue fever occurred in the Seychelles, a group of islands situated north of Madagascar, between India and Africa. This report describes that epidemic, and includes an account of the isolation of dengue type 2 virus, a note on possible vector mosquitos on the main islands, and the results of antibody tests on sera collected towards the end of the outbreak.

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THE SEYCHELLES

The Republic of Seychelles consists of over 100 islands scattered over 400 000 km² of the Indian Ocean between 45° and 60° E and 4° and 11° S. Thirty-two of the islands are granitic with a total land area of 235 km², the rest being coralline, with a land surface of 210 km². Of the total population of 61 900, 88% live on Mahé, the largest of the granitic islands, and a further 11% on the other granitic islands, all of which lie within 55 km of Mahé. The capital and only town is Victoria. Its total population is 23 000, of which 15 600 live in urban and 7400 in rural areas (*4*). The granitic islands are mountainous, the highest peak in Mahé being 905 m above sea level. The coral islands are flat, seldom rising more than 10 m above the sea.

Fourteen species of mosquito are known to be present on Mahé and nearby islands. In numbers, *Aedes albopictus* is predominant, followed by *Culex pipiens fatigans* and *Culex simpsoni*. *A. aegypti* is restricted to a few areas of Victoria only.

THE EPIDEMIC

Cases of a dengue-like illness were first seen on Mahé in December 1976. The number of patients visiting the outpatient clinics with the disease increased rapidly and reached a peak by the end of February, when an estimated 60% of the population had suffered or were suffering from the epidemic disease. The veterinary service reported that no unusual disease or abortions had been observed in domestic animals.

Early during the outbreak the health authorities received reports indicating that the epidemic had spread rapidly to the other inhabited islands.

The epidemic lasted until September 1977. Sporadic cases were observed in 1978, mainly in people who had recently arrived in Seychelles. In September 1978 there was again a small outbreak of dengue-like illness.

Staff of the Ministry of Labour Health and Welfare (MLHW) calculated from the number of absences due to sickness that about 75% of the population of Mahé had suffered from the epidemic disease. On Praslin and La Dique, two other granitic islands, house-to-house surveys were conducted in order to estimate the extent of the epidemic. On Praslin, about 50% of the population were questioned, of whom 75% claimed to have had the disease; on La Dique, 35% of the people were surveyed, of whom 66% seemed to have been ill with dengue. Information received from the other inhabited islands revealed that, in some with small populations, all the people seemed to have been affected. Only a few of the smaller islands escaped the epidemic.

Clinical features

The clinical picture of the epidemic disease was consistent with dengue fever and was characterized by the following signs and symptoms:

- Fever up to 40 °C, associated with headache that was usually severe and mainly frontal in location. Pain on moving the eyes was a prominent feature.
- Myalgia was very common, as was pain in the loins.
- Anorexia, aggravated by a foul taste, was a common complaint.
- Vomiting and diarrhoea were relatively rare symptoms.
- A small number of male patients suffered from orchitis.
- Some of the patients complained of diplopia or blurred vision, others of clumsiness or changes in their ability to do coordinated tasks, such as writing.
- An itchy, punctate, or morbilliform rash, usually of short duration, was seen in many patients.
- Dengue haemorrhagic fever and the dengue shock syndrome were never diagnosed, although some women complained of periods that were heavier than normal.
- In 1975, 1976, and 1978 there were seven deaths each year due to encephalitis, meningitis, and diseases diagnosed as "probably viral". Using the same criteria, in 1977 there were 20 such deaths. This excess mortality may have been due to dengue and its complications.

MATERIALS AND METHODS

On 7 February 1977 12 serum specimens taken from patients in the acute phase of the illness were sent to the virus laboratory of the Medical Research Centre in Nairobi, Kenya, where they were inoculated intracerebrally into suckling mice. After approximately two weeks some of the animals began to show signs of a slowly developing encephalitis. Although the long incubation period and slow development of encephalitis are not pathognomonic for dengue viruses, the signs were consistent with what is commonly seen when the viruses are isolated in these animals, and the illness of the mice was strongly suggestive of the presence of an arbovirus.

During the last week of February, 73 serum samples were collected in clinics in Mahé from patients in the first two days of illness, stored in liquid nitrogen, and taken to Nairobi together with more than 1000 mosquitos and 400 *Leptoconops spinosifrons* (Carter) midges. The arthropods were collected by the MLHW's entomologist and his staff.

The isolates from the first series of 12 specimens were passaged repeatedly in newborn mice for better adaptation. Virus isolation attempts were initiated also with the material taken frozen to Nairobi. Serum specimens and supernatants of centrifuged arthropod suspensions were inoculated in the same way as the first specimens. Two of the isolates from serum were forwarded to the Pasteur Institute at Dakar. From there, after identification as a flavivirus, isolate SS20 was sent to the Yale Arbovirus Research Unit at New Haven, Connecticut, for further analysis. Isolate C1544 was shipped to the Royal Tropical Institute of Amsterdam, and isolate C1538 to the Arbovirus Unit of the London School of Hygiene and Tropical Medicine.

Post-epidemic serum specimens were collected in Mahé and tested for antibodies. Of the 133 sera, 79 were taken in September 1977 and 53 during the first eight months of 1978, before the second small outbreak. The people were not randomly chosen. Portions of serum specimens taken from patients for other reasons were set apart and stored frozen before shipping to Amsterdam for serology. The dengue anamnesis was recorded for each patient, but this history had no influence on selection. There is therefore no reason to suppose that the sample was biased with respect to the prevalence of dengue antibodies. The patients were aged between 6 and 72 years.

Virus isolation in mosquito cells

At the London School of Hygiene and Tropical Medicine, virus isolation was attempted from acute-phase serum specimens in the AP-61 *Aedes pseudoscutellaris* cell line, developed by Varma et al. (*5*).

Monolayers of low passage AP-61 cells were prepared in 25-cm² Corning plastic flasks as described previously for virus isolation (*5*, *6*) and for plaque assay (*7*, *8*). Serum samples were diluted 1:10 in Leibovitz L15 medium with 50 ml of fetal calf serum per litre, and 0.5-ml aliquots were inoculated. Flasks were incubated at 28 °C and observed for 14 days.

Identification

In London, identification of the partly mouse-adapted isolate C1538 was carried out, after 4 more mouse brain passages, by plaque neutralization tests using the AP-61 cells and hyperimmune sera to the alphaviruses Chikungunya (CHIK) and O'nyong-nyong (ONN) and the flaviviruses dengue 1 and dengue 2 at a dilution of 1:10.

At the Royal Tropical Institute of Amsterdam, strain C1544 was identified in a modified plaque reduction test using the method of Early et al. (*9*) and the epitheloid human embryonic lung cell line L-132, developed by Davies (*10*). The isolate was first compared with the prototype New Guinea strain of dengue 2 virus, using rabbit antisera. Subsequently, the two viruses were tested against immune mouse ascitic fluids to the four dengue prototype strains. The fluids were kindly provided by the Center for Disease Control (CDC), Atlanta.

In New Haven, Dr R. E. Shope tested strain SS20 against dengue 2 mouse serum using an indirect fluorescent antibody test.

Serology

In order to obtain information on possible past or present circulation in the Seychelles of flaviviruses that might cross-react with C1544, the post-epidemic sera were also tested against the 17D yellow fever vaccine (YF) and West Nile (WN) viruses. Preliminary experiments showed that for C1544 virus the plaque reduction test gave optimal results; for the two other viruses the tube-neutralization test as described by Hammon & Sather (*11*) was better. Both tests were performed in the L-132 cell line. The lowest dilution used in the plaque reduction test was 1:20, in the tube-neutralization test 1:4.

RESULTS

The 12 acute-phase sera, received in Nairobi early during the outbreak, yielded 9 isolates. Of the second series of 73 serum specimens, 20 were inoculated in mice and some of these were also found to be positive. However, the isolates did not adapt easily to mice. In fact, after more than 10 passages none had adapted sufficiently to give a constant incubation period or 100% mortality. Survivors suffered from chronic encephalitis and hydrocephalus, or showed abnormalities of the fur, similar to those described by Walker (*12*) after inoculation of O'nyong-nyong virus. Attempts to produce a haemagglutinin from the brains failed, probably because of low titres. Shortage of mice and lack of time prevented further attempts at isolation from the sera and the isolates that had apparently adapted best were selected for further study. One of the isolates of the second series was the SS20 strain.

A total of 24 second series' specimens were tested in the AP-61 cell line and 14 isolates (58%) were obtained. None gave the typical syncytial response as observed with the dengue 2 New Guinea strain (*13*). Occasionally, small syncytial patches were seen by the second or third passage, but often the cells appeared dark and retracted compared with the controls. It is possible that further strains might have been isolated if passages with a few doubtful isolates had been continued. The results of the isolation attempts in cultured mosquito cells suggested that the proportion of positives in the second series of sera was of the same order as that in the first series. The isolates were not typed.

The partly mouse-adapted C1538 strain produced a syncytial type response in the AP-61 cells at dilutions of 10^{-2} and 10^{-3} after 3 days at 32 °C or 4–5 days at 28 °C. Plaques were obtained at dilutions down to 10^{-6} at 28 °C. There was no plaque reduction with CHIK and ONN antisera, whereas more than 99% reduction was obtained with anti-dengue 2 antisera on two occasions. Three neutralizations against dengue 1 antiserum produced negative or insignificant results.

The results of the plaque reduction tests performed in Amsterdam with strain C1544 against two rabbit antisera and four CDC mouse immune ascitic fluids are given in Table 1. C1544 was also clearly identified as a type 2 dengue virus strain.

Table 1. Results of plaque-reduction tests for C 1544 and dengue 2 viruses against anti-dengue rabbit sera and mouse immune ascitic fluids

		Dengue 2 (New Guinea)[a]	C 1544[a]
RAS[b]	dengue 2 (New Guinea)	320	> 320
	C 1544	80	160
MIAF[c]	dengue 1 (Hawaii)	40	40
	dengue 2 (New Guinea)	> 640	> 640
	dengue 3 (H-87)	10	40
	dengue 4 (H-241)	< 10	10

[a] Highest serum dilution that produced 50% reduction of plaques.
[b] Rabbit antisera.
[c] Mouse immune ascitic fluid.

In New Haven the SS20 isolate reacted positively with dengue 2 mouse serum in the indirect fluorescent antibody test.

Five of 23 *A. albopictus* pools, 2 of 8 *C. pipiens fatigans* pools and, surprisingly, 1 of 2 *Leptoconops* pools yielded virus. The strains were not identified, but their effects in mice were identical with those of the dengue 2 isolates from serum.

While the work on adaptation and identification of the isolates was underway, 4 strains of dengue 2 virus were isolated in the laboratory of the Pacific Research Unit at Honolulu, Hawaii, from serum of United States Navy personnel who had returned from the Seychelles (L. Rosen, personal communication, 1979). In Hawaii, a rapid method was used, in which serum is inoculated into the thorax of the large *Toxorhynchitis amboinensis* mosquito (*14*). Infected mosquitos are identified by immunofluorescence (*15*) and the virus is typed by complement fixation (*16*).

Of 133 post-epidemic sera, 100 (75%) reacted with dengue type 2 antigen, 18 (14%) had antibodies against WN virus, and 31 (23%) against YF virus. When the sera were divided according to age of donor (Table 2) it was evident that all age groups had been equally exposed to dengue 2 virus, although there was a slightly higher prevalence of antibodies in the group over 40 years of age.

Antibody titres were lowest for WN virus. Only one serum had a titre of 16. In 17 of the 18 WN-positive sera the titres were less than one-quarter of those for either dengue (16 cases) or YF (1 case) in the same serum. Only one serum had a titre of 8 for WN antibodies in the absence of antibodies to the other two viruses. The serum belonged to the series collected in September 1977 and came from a patient who at the time of giving a blood sample had a fever clinically diagnosed as dengue. We cannot offer more than speculative explanations for this observation. The generally low titres for WN antibodies and the regular association with antibodies against one or both of the other viruses, suggest that the antibodies cross-react and that WN virus itself did not play a role in their production. The higher proportion of positives for WN antibodies in the oldest age group may be explained by an earlier exposure of this group to a dengue virus.

Table 2. Distribution of antibodies to dengue 2, yellow fever, and West Nile viruses

Age group (years)	Dengue 2		Yellow Fever		West Nile	
	No. positive/ No. tested	%	No. positive/ No. tested	%	No. positive/ No. tested	%
< 20	23/30	77	4/30	13	3/29	10
20 - 29	25/35	71	5/35	14	2/34	6
30 - 39	22/31	71	10/31	32	2/30	7
⩾ 40	30/37	81	12/37	32	11/36	31
Total	100/133	75	31/133	23	18/129	14

Table 3. Relation between dengue and yellow fever antibody titres in 31 sera

Yellow fever antibody titre	Dengue antibody titre			
	< 20	20 - 40	160 - 320	640
4 - 8	2	11	0	1
16 - 32	2	6	1	1
64	1	2	0	0
⩾ 128	0	2	1	1

The antibody titres for YF and dengue 2 viruses are given in Table 3. Some sera were positive for YF and did not react with dengue 2 antigen at a dilution of 1:20. Some sera showed higher titres for YF than for dengue 2 antibodies. This observation and the higher prevalence of antibodies in the group over 40 years of age (Table 2) can largely be explained by the assumptions that the YF antibodies in some people are the result of vaccination against YF, and in others, of cross-reactions with dengue 2 antigen. The possibility that still another flavivirus played a role cannot be altogether excluded, but there is little evidence to support this.

It is therefore highly likely that the vast majority, if not all, of the dengue antibodies found in the post-epidemic sera resulted from infections with dengue virus.

Table 4 shows the dengue 2 antibody titres separately for persons below and over 40 years of age. Most of the high titres were found in the group of older subjects. The means of the titres in the two groups are 60 and 256, and the distribution of titres in the two groups was found to be significantly different ($P < 0.001$).

In Table 5 the antibody titres of the 133 post-epidemic sera are related to the history of dengue as reported by the patient at the time the blood samples were taken. For 87 cases the two indicators are in agreement, but for 46 subjects the results are contradictory. Seven people, who stated that they had not suffered from dengue, had titres between 320 and 2560. Six of these were over 51 years of age and one was 9 years old. Only one older person, aged 51 years, whose titre was 320, claimed to have had dengue. The relatively frequent association in older people of high titres for dengue 2 antibodies and apparently sub-

Table 4. Distribution of dengue 2 neutralizing antibody titres in 133 post-epidemic sera

Age group (years)	No. examined	Antibody titre								
		< 20	20	40	80	160	320	640	1280	≥ 2560
< 40	96	26	29	30	4	3	3	1	0	0
≥ 40	37	6	8	8	2	5	4	2	1	1
Total	133	32	37	38	6	8	7	3	1	1

Table 5. Correlation between anamnesis and serological test results

Antibody titre	Anamnestic report					
	Positive		Negative		Total	
	No.	%	No.	%	No.	%
≥ 320	5	6	7	15	12	9
80 - 160	11	13	3	7	14	11
20 - 40	55	63	20	43	75	56
< 20	16	18	16	35	32	24
Total	87	100	46	100	133	100

clinical infections suggests that some of them had had antibodies to the virus before the epidemic, which had been boosted by recent infection. This suggests that the Seychelles experienced an epidemic of dengue fever approximately 50 years ago, which would partly explain the discrepancy between the serological results and the anamnesis.

Some of the other discrepancies may have resulted from the inevitable mistakes that are made with anamnestic questioning. Nevertheless, there is a significant difference between the antibody titres found in persons with and without a recent history of dengue fever ($P < 0.02$).

DISCUSSION

Between December 1976 and September 1977 the Republic of Seychelles was struck by an unusually extensive epidemic of dengue fever. In four laboratories seven different isolates were identified as dengue virus type 2, which was apparently responsible for the outbreak.

The MLHW had records of a large epidemic diagnosed as dengue, which started in Seychelles in June 1926 and continued for about a year. If cases continued to occur for another ten years, it would explain the high antibody titres in older people, which presumably resulted from boosters by recent infections. High titres produced by booster effects following subsequent infections by two of the closely related types of dengue virus are common. The 1926 epidemic may therefore have been caused by any of the four dengue virus types.

Clinically the epidemic disease had the characteristics of classical dengue fever. Orchitis has been observed during other epidemics (*17*). Fatal encephalopathy, confirmed by virus isolation, was reported from Indonesia by Sumarmo et al. (*18*). It seems possible therefore that at least some of the cases that contributed to the high mortality rate in 1977 were associated with the dengue epidemic.

The mosquitos *A. albopictus* and *C. pipiens fatigans* were present in large numbers near human habitations. Virus was isolated from pools of both species. In itself this is no proof of their role as vectors of the virus, and Theiler & Downs (*19*) state that in experiments *C. fatigans* failed to transmit dengue viruses. The isolates made from the Seychelles specimens may therefore have come from recent bloodmeals on viraemic patients. The same may be true for the isolate from *Leptoconops* midges, although experiments to test their potential as a vector would be of interest. However, *A. albopictus* is a known vector of dengue viruses, and Gubler & Rosen have shown that the agents replicate to high titres in this species (*20, 21*). The mosquito transmitted dengue virus in experiments (*22*) and dengue 2 virus was isolated from wild specimens caught by Gould et al. (*23*).

In most parts of the world the principal vector of dengue virus is the ubiquitous *A. aegypti* mosquito, but on Mahé it cannot have played more than a minor role because of its limited distribution. In the authors' opinion there need be little doubt that *A. albopictus* was responsible for the greater part, if not all, of the transmission during the epidemic in the Seychelles.

If this were the case, it would not be a unique occurrence. In Japanese cities there were outbreaks of *A. albopictus*-transmitted dengue between 1942 and 1945 (*24*), and there have recently been two more similar outbreaks (L. Rosen, personal communication, 1979).

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RÉSUMÉ

ÉPIDÉMIE DE DENGUE TYPE 2 AUX SEYCHELLES, PROBABLEMENT TRANSMISE PAR *AEDES ALBOPICTUS* (SKUSE)

En décembre 1976, une vaste épidémie de dengue a commencé aux Seychelles, groupe d'îles de l'océan Indien. Elle a atteint son maximum au cours de la dernière semaine de février 1977, puis a décliné petit à petit pour se terminer virtuellement en septembre. Quelques cas sporadiques ont été diagnostiqués en 1968, et en septembre de cette même année s'est produite une deuxième petite épidémie. Le tableau clinique était celui de la dengue classique et il n'a pas été observé de dengue hémorragique ou de syndrome de choc de la dengue.

Au point de vue quantitatif, *A. albopictus* est le principal moustique de ces îles, suivi par *Culex pipiens fatigans*. *A. aegypti* se rencontre seulement dans de rares secteurs de Victoria, la capitale de la République des Seychelles, et dans quelques îles coralliennes.

Le virus de la dengue type 2 a été isolé des malades, de *A. albopictus* et de *C. fatigans*. Chez ce dernier, la présence du virus s'expliquait vraisemblablement par le fait que le moustique s'était gorgé récemment sur un malade virémique, et non par une multiplication, car *C. fatigans* n'est pas considéré comme un vecteur des virus de dengue. *A. albopictus* était le vecteur probable au cours de l'épidémie. Des calculs fondés sur l'absentéisme dans les principales îles, les questionnaires anamnéstiques dans d'autres îles et le résultat de la recherche des anticorps dans 133 sérums recueillis sur l'île principale après la fin de l'épidémie, donnent à penser qu'une grande majorité de la population (près de 75%) avait été touchée.

Une épreuve de réduction des plages a été utilisée pour l'enquête sérologique. En ce qui concerne l'anticorps anti-dengue 2, il a été mis en évidence dans 100 des 133 sérums mais il n'y avait pas concordance entre le titre de l'anticorps neutralisant et l'anamnèse. En effet, sur les 12 sujets ayant des titres allant de 320 à 2560 et plus, 7 ont nié un épisode récent de dengue. Sur ces 7 personnes, 6 avaient plus de 51 ans, le dernier étant un enfant de 9 ans. L'association relativement fréquente, chez les personnes les plus âgées, de titres élevés d'anticorps anti-dengue 2 et d'infection apparemment infraclinique donne à penser que certaines d'entre elles possédaient déjà des anticorps avant l'épidémie, l'infection récente ayant eu un effet de rappel. On a d'ailleurs découvert qu'il y avait eu aux Seychelles, en 1926–1927, une maladie pour laquelle le diagnostic de dengue avait été porté. Cela expliquerait partiellement les discordances entre les résultats sérologiques et l'anamnèse. La récente épidémie des Seychelles a révélé, au-delà de tout doute raisonnable, les capacités de *A. albopictus* de transmettre le virus de la dengue.

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