Myocarditis and cardiomyopathy after arbovirus infections (dengue and chikungunya fever)

Ivor Obeyesekere and Yvette Hermon

From the Cardiology Unit, General Hospital, Colombo, and the Virus Laboratory, Medical Research Institute, Colombo, Ceylon

The varying clinical features and sequelae in 10 patients suffering from myocarditis after dengue and chikungunya fever caused by arboviruses are described. These patients had significantly raised antibody levels in the serum and gave a recent history of ‘dengue-like’ fever.

A few patients had a favourable outcome with disappearance of symptoms, improvement in the electrocardiogram, and no residual cardiomegaly. Others had persistent symptoms, electrocardiographic changes, and cardiomegaly which suggested a transition to cardiomyopathy, a chronic cardiac disorder. It seems likely that arbovirus infections play a significant role in the aetiology of the cardiomyopathies which are common in Ceylon.

Myocarditis or inflammation of the myocardium refers to myocardial lesions and symptoms associated with infectious diseases and specific infections. Among the reported causes are bacterial, viral, mycotic, protozoal and Rickettsial infections, parasitic infestations, and specific bacterial toxins. Of the viruses, Coxsackie B, and less frequently viruses causing infectious mononucleosis, infectious hepatitis, mumps, measles, poliomyelitis, aseptic meningitis, and encephalo-myocarditis have been reported to be pathogenic to man. While dengue and chikungunya fever are well recognized, there are very few published reports concerning their cardiac complications (Boon and Tan, 1967; Hyman, 1943). Myocarditis, which was thought to be benign, is now known to lead to a more chronic disorder, cardiomyopathy (Bengtsson, 1968).

This paper describes the varying clinical features and sequelae of patients suffering from myocarditis in association with dengue and chikungunya fever caused by arboviruses and referred to as ‘arbovirus myocarditis’.

Subjects and methods

For the purpose of this study 10 patients were selected in whom a diagnosis of arbovirus myocarditis was made. The following diagnostic criteria were used: (1) Clinical evidence of myocarditis; (2) the presence of electrocardiographic evidence of myocarditis, ST segment and T wave changes, and disturbances in conduction and rhythm; (3) a recent history of ‘dengue-like’ fever; (4) serological evidence of past dengue or chikungunya infection as revealed by the presence of antibody in high titre.

The study included the history, physical examination, serial electrocardiograms, serial x-rays of chest, and cardiac catheterization in two patients. The laboratory tests included routine examination of urine and blood, estimation of transaminases, erythrocyte sedimentation rates, complement-fixation tests for toxoplasmosis and filariosis, antistreptolysin titres, and tests for systemic lupus erythematosus.

Serological tests for arbovirus antibody were carried out by means of the haemagglutination-inhibition test to determine evidence of past arbovirus infection. Simultaneously, the same test was carried out on 3 control groups of patients also admitted to the Cardiology Unit: (1) those with rheumatic heart disease, (2) those suffering from coronary heart disease, with characteristic electrocardiographic changes and transient rises in activity of serum enzymes (SGOT), and (3) those with congenital heart disease. The complement-fixation test was done on 10 patients suffering from myocarditis who showed high haemagglutination-inhibition antibody levels. The description of the antigens used and serological techniques are described in the Appendix.

Results

The results of haemagglutination-inhibition and complement-fixation tests in 10 patients with myocarditis are given in Table 1. All these (except Case 9) had haemagglutination-inhibition antibody levels of 1:640 or greater to either dengue (Type I or II) or chikungunya, and all (except Case 1) had comple-
ment-fixation antibody levels of 1:64 or greater. Ten other patients with myocarditis had haemagglutination-inhibition titres of 1:320 and above to dengue or chikungunya.

Table 2 gives the haemagglutination-inhibition and complement-fixation test results in the 3 control groups. There was a striking and highly significant difference, with only one patient with a haemagglutination-inhibition titre of 1:320 and two with titres of 1:160.

A serological diagnosis of arbovirus infection necessarily depends on the examination of paired sera, the first sample being collected in the acute phase and the second convalescent sample 2 or 3 weeks later. The criteria for serological diagnosis have been described (Nimmannitya et al., 1969). Primary and secondary or recurrent infection are recognized according to the haemagglutination-inhibition and complement-fixation antibody responses. In the 10 patients described in this paper, a diagnosis of acute arbovirus infection was not initially sought. By the time these patients presented themselves at the cardiology unit they already had established cardiac disorders which followed recent ‘dengue-like’ illnesses. Five of these patients (Cases 1, 5, 6, 7, and 8) also gave definite histories of similar attacks of fever occurring months or years earlier. Only evidence of past arbovirus infection was, therefore, sought and a single sample of blood was sent for detection of arbovirus antibody. The results revealed that all these patients had high dengue and chikungunya haemagglutination-inhibition and complement-fixation antibody which confirmed a past arbovirus infection. It is postulated that dengue and chikungunya fevers, with their prominent myalgic manifestations, involved the myocardium and triggered off the chain of symptoms resulting in acute myocarditis followed, in some cases, by cardiomyopathy.

All the 10 patients with acute myocarditis gave a history of 'dengue-like' illness in the recent or distant past. It was not possible by determining the haemagglutination-inhibition antibody levels of single sera to determine whether the infections were primary or secondary dengue. However, the complement-fixation test results confirmed that all these were secondary or recurrent infections as there was antibody to both Types I and II dengue. Two patients (Cases 1 and 6) showed high haemagglutination-inhibition and complement-fixation titres to chikungunya as well as dengue haemagglutination-inhibition and complement-fixation antibody, which indicated infections with both viruses in the past. The main clinical features and sequelae of 10 patients with arbovirus myocarditis are illustrated by case histories.

**Case histories**

**Case 1** A 43-year-old housewife developed sharp pain over the praecordium, difficulty in taking a deep breath, profuse sweating, and dizziness.

A few days previously and also three months before she had had fever with recurrent headaches, backache, and pains in the joints.

On examination, temperature was 37.4°C, pulse rate 78 a minute with frequent ectopics, and
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The blood pressure was 130/70 mmHg. Both heart sounds were heard. Crepitations were heard at the base of the left lung. She ran a low temperature for five days and complained of extreme fatigue and breathlessness. The ventricular ectopies persisted for four weeks.

Two months later, she was symptom free; there were no abnormal physical signs in the heart.

Comments
This woman presented with severe chest pain simulating coronary heart disease. She had extensive electrocardiographic changes, a raised erythrocyte sedimentation rate, normal serum enzymes, and positive serology for arbovirus infection. The pain was probably pericardial in origin. She made a satisfactory recovery within two months with no symptoms, return of the electrocardiogram to normal, and no residual cardiomegaly.

Case 2 An unmarried woman aged 24 years presented with fever of 10 days' duration, generalized aches and pains, painful joints, palpitations, and sharp pain over the precordial made worse by deep breathing.

On examination, she looked ill and pale, respirations were rapid, temperature was 40°C, pulse 124 a minute and irregular. Blood pressure was 110/80 mmHg. On auscultation there was a gallop rhythm with a prominent third heart sound, and soft systolic murmur over the mitral area.

Six months later she had remained well apart from a relapse of fever and tachycardia. She complained of slight breathlessness on effort and occasional palpitations. The pulse rate was 124 a minute and blood pressure 110/60 mmHg. On auscultation there was gallop rhythm with a prominent third heart sound.

Comments
This woman presented with high fever, chest pain, difficulty in breathing, an irregular pulse due to AV dissociation, and atrial ectopy and gallop rhythm. Six months later she continued to have mild symptoms and the electrocardiogram showed sinus tachycardia; the heart size was normal and the gallop rhythm persisted.

Case 3 A married woman aged 33 years complained of increasing fatigue, headache, dyspnoea on exertion, and dizziness of six weeks' duration after a low fever with generalized aches and pains. On examination, pulse was 72 a minute, with occasional ventricular ectopies, jugular venous pressure normal, blood pressure 160/80 mmHg. On auscultation both heart sounds were heard; there were no added sounds. While in hospital she had a recurrence of fever.

Six months later she complained of dyspnoea, and undue fatigue on effort. Pulse rate was 72 a minute, blood pressure 130/80 mmHg, and heart sounds were normal.

| Table 2 Virus serological results of patients with (a) rheumatic heart disease, (b) coronary heart disease, and (c) congenital heart disease (controls) |
| Disease | Control No. | Age | Sex | Haemagglutination inhibition titre |
|---------|-------------|-----|-----|-----------------------------------|
|         |             |     |     | JF | D1 | D2 | Chik |
| Rheumatic heart disease (12) | 1 | 20 | F | <20 | 80 | <20 | <20 |
| Coronary heart disease (8) | 2 | 12 | F | <20 | <20 | <20 | <20 |
|       | 3 | 10 | F | <20 | <20 | <20 | <20 |
|       | 4 | 41 | F | <20 | 80 | 40 | <20 |
|       | 5 | 10 | F | <20 | <20 | <20 | <20 |
|       | 6 | 24 | F | <20 | <20 | <20 | <20 |
|       | 7 | 17 | F | <20 | <20 | <20 | <20 |
|       | 8 | 33 | F | <20 | <20 | <20 | <20 |
|       | 9 | 22 | M | <20 | <20 | <20 | <20 |
|       | 10 | 19 | M | 40 | 320 | 320 | 20 |
|       | 11 | 26 | F | <20 | <20 | <20 | <20 |
|       | 12 | 17 | F | <20 | 80 | 40 | <20 |
| Congenital heart disease (4) | 13 | 45 | M | <20 | <20 | <20 | <20 |
|       | 14 | 48 | M | <20 | <20 | <20 | <20 |
|       | 15 | 45 | M | 80 | 40 | 80 | <20 |
|       | 16 | 48 | M | 80 | 160 | 80 | <20 |
|       | 17 | 57 | F | 80 | 80 | 160 | 160 |
|       | 18 | 44 | M | <20 | <20 | <20 | <20 |
|       | 19 | 45 | M | <20 | <20 | <20 | <20 |
|       | 20 | 35 | M | 80 | 80 | 80 | <20 |

Comments
This patient, who gave a history of six weeks' disability following low grade fever, had occasional ventricular ectopies, an abnormal electrocardiogram, radiographic evidence of cardiomegaly, and very high dengue antibody titre. The persisting electrocardiographic changes and cardiomegaly suggest transition from acute myocarditis to cardiomyopathy.

Case 4 A married woman aged 48 years was first seen in March 1971 with a history of chest pain accompanied by difficulty in breathing, sweating, and fainting.

In November 1970 she had dengue fever, 3 months after which she had recurrent substernal chest pain, palpitations, and breathlessness on exertion, which progressively increased.

On examination she looked anxious and pale, the pulse was 96 a minute, blood pressure 110/80 mmHg, and there was a gallop rhythm heard with a prominent third heart sound.

Six months later she continued to have breathlessness, chest pain, and fatigue on undue exertion.

Comments
This 48-year-old woman gave a history of dengue fever. She continued to have persistent mild symptoms, electrocardiographic changes, and cardiomegaly suggesting cardiomyopathy. Her
5-year-old daughter (Case 10) who gave a similar history had a high titre of antibodies for dengue and changes in the electrocardiogram.

Case 5 A married housewife aged 32 years was first seen in February 1971 complaining of recurrent chest pain, breathlessness, and fatigue on mild exertion.

In early December 1970, she had high fever of sudden onset accompanied by headache, backache, painful joints, and sharp substernal chest pain. A month later she had a relapse of fever and more severe chest pain, during which she blacked out, sweated, vomited, and felt dizzy. She was treated for 'benign pericarditis'. She continued to have substernal discomfort on exertion, palpitations, mild breathlessness, and undue fatigue.

On examination, pulse rate was 88 a minute with occasional ectopics, and blood pressure was 90/70 mmHg. There was a distinct third heart sound.

Six months later she continued to have symptoms; the cardiac enlargement and electrocardiographic changes persisted.

Comments
This 32-year-old woman with a well-documented history of 'benign pericarditis' and a positive serology for dengue continued to have symptoms, persistent cardiomegaly, and electrocardiographic changes suggesting cardiomyopathy.

Case 6 A woman aged 44 years, mother of 10 children, was admitted to hospital in February 1971, with a history of increasing breathlessness on exertion, nocturnal dyspnoea, dizziness, and undue fatigue of 6 weeks’ duration, after a 'dengue-like' fever.

On examination, she was mildly orthopnoeic, pulse rate was 92 a minute and irregular, jugular venous pressure was raised 4 cm, and blood pressure was 160/100 mmHg. On auscultation there was a gallop rhythm with prominent third heart sound. There were scattered ronchi and crepitations heard over both lung bases. The liver was palpable 4 cm.

She had a relapse of fever in hospital. Treated with strict bed rest for four weeks, she became free of symptoms; pulse rate was between 68 to 80 a minute, blood pressure was normal, and the adventitious sounds in the lung cleared. She had a relapse of fever three months later with worsening of symptoms.

Six months later she had signs of early congestive failure, sinus tachycardia (rate 120 a minute), raised jugular venous pressure, a blood pressure of 150/90 mmHg, gallop rhythm with prominent third heart sound, hepato-megaly, and ankle oedema.

Comments
This multiparous female presented with impaired effort tolerance and nocturnal dyspnoea after chikungunya fever. She had sinus tachycardia, frequent ventricular ectopics, gallop rhythm, cardiomegaly, and an abnormal electrocardiogram. Six months later she had signs of congestive cardiac failure. She provides an example of congestive cardiomyopathy after chikungunya fever.

Case 7 A male medical colleague was aged 32 years when first seen in 1953 with recurrent chest pain and palpitations after a 'dengue-like' fever. He had an irregular pulse due to frequent ventricular ectopics. Virus serology was not done. Electrocardiogram showed non-specific T wave inversion over leads II, III, aVF, and V5-V6. He suffered from palpitations for many years after this but continued to lead an active life. The ventricular ectopics and the electrocardiographic changes persisted with a deep S in V2 (30 mm) and prominent R in V5 (25 mm) wave. Teleradiogram confirmed the presence of cardiomegaly.

In January 1971 after an attack of dengue fever he noticed increasing breathlessness on exertion, ankle oedema, effort syncope, dizzy spells, and easy fatigue. On examination jugular venous pressure was raised, pulse 40 a minute, and blood pressure 160/80 mmHg. There was a loud late systolic murmur over the mitral area. There were crepitations at both lung bases; and hepatomegaly and ankle oedema were present.

Six months later, he continued to have symptoms and manifested evidence of early congestive heart failure, persisting cardiomegaly, and an abnormal electrocardiogram.

Comments
A 32-year-old medical colleague developed cardiomegaly and an abnormal electrocardiogram (cardiomyopathy) after a 'dengue-like' fever in 1953. The abnormal electrocardiogram and cardiomegaly remained unchanged. He continued to lead an active but sedentary life for 18 years. After an attack of dengue fever in 1971 he developed cardiac failure with evidence of congestive cardiomyopathy.

Case 8 A 16-year-old girl was first seen in 1968 with a history of increasing breathlessness, chest pain, palpitations, and oedema of vulva and both lower limbs.

In 1962 she had a 'dengue-like' fever, painful swelling of knees and ankle joints, after which she developed mild exertional dyspnoea. She had recurrent attacks of fever between 1962 and 1968. She had no cardiac murmurs and the ASO titre was repeatedly negative.

On examination she was underweight and mildly orthopnoeic, pulse rate 84 a minute, pulsus paradoxus was present, the jugular venous pressure raised 14 cm, and blood pressure 100/80 mmHg. The cardiac impulse was not palpable. There was wide splitting of the pulmonary second sound and a gallop rhythm with a loud third heart sound. There was a right-sided pleural effusion, palpable liver (8 cm), and generalized anasarca, with oedema of the face and ascites.

She was treated with digitalis and diuretics.
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With time she became progressively breathless, and her generalized oedema became more difficult to control necessitating larger doses of diuretics.

Comments
This young girl, who gave a history of recurrent dengue, developed evidence of cardiac constriction confirmed by cardiac catheterization.

Case 9  A 46-year-old bus driver was referred for investigation because of an irregular pulse. Three months previously he had had high fever and then noticed breathlessness after severe exertion. He smoked 20 cigarettes daily and consumed 1 bottle arrack daily for 5 years. On examination he was underweight, pulse rate was irregularly irregular, rate 88 a minute, and blood pressure was 110/70 mmHg. There was a gallop rhythm with a prominent third heart sound best heard over the mitral area.

Comments
Employees of the Ceylon Transport Board are examined periodically. This man was quite normal until he developed fever 3 months previously, after which he developed atrial fibrillation and cardiomegaly. The positive viral serology indicated recent dengue and chikungunya fever.

The persisting cardiomegaly and arrhythmia with no evidence of heart failure suggests transition from acute myocarditis to 'precongestive' cardiomyopathy. His heavy intake of alcohol may have contributed.

Case 10  This 5-year-old daughter of Case 4 developed a 'dengue-like fever' with her mother in November 1970, complaining of severe chest pain and difficulty in breathing. She continued to be listless, easily tired, lethargic, and disinclined to play for six months. On examination in May 1971 she looked pale and underweight. Pulse was 100 a minute and blood pressure 90/70 mmHg, heart sounds were normal.

Comments
This 5-year-old daughter of Case 4 developed dengue fever and symptoms of acute myocarditis. She had persistent electrocardiographic changes and serology positive for dengue fever.

Laboratory investigations  The erythrocyte sedimentation rate done on 9 patients was raised with readings in the first hour between 20 and 40 mm in 5, between 40 and 90

| Case No. | Time of investigation | Electrocardiograms | Radiology of heart |
|----------|-----------------------|--------------------|--------------------|
| 1        | On admission          | Low voltage, frequent ventricular extrasystoles, ST\(_d\) and T\(_d\) II, III, aVF, V1-V6 | Cardiothoracic ratio 0.42 |
| 2        | On admission          | Heart rate 128/min; AV dissociation; frequent atrial extrasystoles |                  |
| 3        | On admission          | Heart rate 118/min; 1st-degree heart block; PR interval 0.24 sec |                  |
| 4        | On admission          | Heart rate 100/min; sinus rhythm; PR interval 0.18 sec |                  |
| 5        | On admission          | Heart rate 100/min; sinus rhythm |                  |
| 6        | On admission          | Low voltage, T\(_d\) or flat I, II, III aVF aVF V1-V6 |                  |
| 7        | On admission          | Sinus rhythm; T\(_d\) I, aVL, V1-V6 |                  |
| 8        | On admission          | Sinus rhythm; T\(_d\) I, aVL, flat T, V5-V6 |                  |
| 9        | On admission          | Sinus rhythm; T\(_d\) I, aVL, flat T, V5-V6 |                  |
| 10       | On admission          | Sinus rhythm; T\(_d\) V1-V4 |                  |

ST\(_d\) = ST segment depression; T\(_d\) = T wave inversion.
mm in 2, and above 90 mm in 2. The haemoglobin levels ranged between 9.6-13.7 g/100 ml. Serum aspartate aminotransferase (SGOT) and antistreptolysin titre were within normal limits. Blood for antinuclear factor, microfilariae, and haemagglutination test for toxoplasmosis were negative. Blood picture studies were normal.

Electrocardiograms The electrocardiograms (Table 3) showed rhythm disturbances including sinus tachycardia, sinus bradycardia, atrioventricular conduction disturbances, and atrial fibrillation. Atrial and ventricular ectopic beats were common in the acute phase and during convalescence. T wave abnormalities and ST segment changes were commonly encountered indicating focal myocardial damage.

Radiology of the heart The cardiothoracic ac ratios determined from serial radiographs of the chest are described in Table 3. Cardiomegaly was common and often persisted long after the acute illness was over.

Cardiac catheterization Right heart catheterization was done on Case 6, two weeks after bed rest and treatment. The haemodynamic findings were normal: (pressure in mmHg), right atrial pressure (mean) 2, right ventricle 30/0 (end-diastolic 2), pulmonary artery 30/8, mean 16, pulmonary capillary wedge (mean) 6. Cardiac output was 6.2 l.

The findings at right and left heart catheterization and cineangiography on Case 8 in October 1968 are shown in Table 4.

Cineangiography with injection of contrast into the left ventricular cavity showed a mild degree of mitral incompetence with thickening of the left ventricular wall. The left ventricle emptied satisfactorily.

Discussion Dengue fever, caused by a group B arbovirus, has been endemic in Ceylon for many years (Mendis, 1967), and dengue virus (Type I) has been isolated (Hermon, Anandarajah, and Pavri, 1970). Chikungunya fever, which is caused by a group A arbovirus, appeared in epidemic proportions in 1965 and was confirmed virologically (Hermon, 1967). A haemagglutination inhibition serological island-wide survey carried out in 1966–67 revealed that group B arbovirus infections were endemic throughout the island, while chikungunya infections were less prevalent (Vesenjak-Hirjan, Hermon, and Vitarana, 1969). From the antibody levels obtained in the island survey haemagglutination inhibi-

| Site                        | Oxygen saturation (%) | Pressure (mmHg) | Mean |
|-----------------------------|-----------------------|----------------|------|
| Superior vena cava          | 76                    | 16, 13, 16, 11 | (14) |
| Right atrium                | 76                    | 16, 13, 16, 11 | (14) |
| Right ventricle             | 76                    | 38/6 (16)*     |      |
| Pulmonary capillary wedge   | 93                    | 18/14          | (16) |
| Left ventricle              | 93                    | 96/8 (16)*     |      |
| Aorta                       | 93                    | 96/80          | (88) |

* End-diastolic pressures.

**Table 4 Findings at right and left heart catheterization in Case 8**

**Determination** titre of 1:640 or greater was arbitrarily fixed as indicative of recent past infection or reinfection. This conclusion was supported by workers in Thailand (Udomsakdi and Halstead, 1966) who reported that 4 months after infection the titre were 1:480 and complement-fixation titres 1:32. All the patients with myocarditis reported in this study had evidence of recent arbovirus infection, suggesting a cause-and-effect relation between infection and cardiac disorder.

It is important to recognize that the heart can be affected since these fevers are extremely common in certain areas, with an increasing incidence. An appreciable number of patients have persistent symptoms, cardiomegaly, and electrocardiographic changes long after the initial illness, giving rise to the cardiomyopathy has been forgotten.

Viruses may invade the myocardium and directly damage the muscle fibres or give rise to a hypersensitivity or autoimmune reaction causing myocardial damage. Moreover, this altered state of the myocardium may persist long after the initial virus infection is over and make it prone to recurrent damage from other agents.

The clinical features in myocarditis are often so vague and non-specific that unless one is aware of its existence in relation to a particular infection, it can often be missed. The signs may be minimal and limited to an irregularity in heart rhythm or minor changes in the electrocardiogram. It may be mistaken for another disease. Two patients complained of severe chest pain which simulated coronary heart disease (Cases 1 and 4). Another had progressive symptoms which led to congestive cardiac failure with cardiomegaly, gallop rhythm with slight rise of blood pressure, and was mistaken for hypertensive cardiac failure (Case 6).

Early on in the disease, the erythrocyte sedimentation rate was raised. The serum enzymes were normal and, being negative, helped to exclude acute myocardial infarction.
Often the earliest clue was provided by abnormalities in the electrocardiogram, multiple ectopics, ST segment and T wave changes. These non-specific changes had to be differentiated from similar changes caused by coronary heart disease, digitalis intoxication, abnormalities after electrolyte disturbances, and hyperventilation due to anxiety. Serial changes in the electrocardiogram were often the best guide to prognosis.

Cases 1, 2, and 10 had a favourable outcome, with gradual disappearance of symptoms, improvement in the electrocardiogram, and no residual cardiomegaly. Cases 3, 4, and 5 became symptom free but had permanent changes in the electrocardiogram and persisting cardiomegaly developing 'precongestive' cardiomyopathy. These patients are liable to relapses and subsequent deterioration. In Case 7 this occurred 18 years after the original attack which, too, was personally treated by one of us (I.O.). Case 6 had persistent symptoms, probably as a result of recurrent subclinical infections, developed cardiomegaly with heart failure, and congestive cardiomyopathy. Case 8 developed signs of right heart failure and evidence of cardiac constrictio. Case 9 developed an arrhythmia, atrial fibrillation, and cardiomegaly.

Arbovirus myocarditis was thus seen to lead to cardiomyopathy with eventual impairment of cardiac function. Cardiomyopathy is a common disorder in Ceylon (Obeyesekere, 1968). It seems likely that arbovirus infections play a significant role in its etiology.

Appendix

Virus serology Antigen: The prototype antigens used for the serological tests were as follows. Japanese encephalitis virus (JE), Nakayama strain; dengue type 1 virus (D1), Hawaiian strain; dengue type 2 virus (D2), New Guinea C strain; and chikungunya virus (Chik), S.27 strain.

These antigens (obtained from Professor Lim Kok Ann of the Virus Laboratory, University of Singapore) were prepared from infected suckling mouse brain, by sucrose acetone extraction, and lyophilized in aliquots of 0.4 ml.

Haemagglutination-inhibition test The haemagglutination-inhibition test was performed by the method of Clarke and Casals (1958), with modifications for micro-titre equipment (Sever, 1962). Eight haemagglutinating units of virus antigen were used in the test, and the patients' sera were diluted in serial twofold dilutions from 1:20 to 1:2560. Each serum, before the test, was absorbed with kaolin and goose cells to remove non-specific inhibitors of haemagglutination. The antigen/serum mixtures were incubated at +4°C overnight, before the addition of goose cells; the plates were then incubated for one hour at room temperature before the tests were read. The haemagglutination-inhibition titre was taken as the last serum dilution at which complete inhibition of haemagglutination occurred.

Complement-fixation test The complement-fixation test was carried out by the Fulton and Dumbell technique (1949) with microtitre modifications, using plastic plates. Two units of complement and 4 units of antigen were used, and the serum/antigen mixtures were incubated at +4°C overnight before addition of the haemolytic system. Each patient's serum was diluted in serial twofold dilutions from 1:4 to 1:256. The complement-fixation titre was taken as the highest dilution of serum showing 75 per cent fixation of cells.

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Requests for reprints to Dr. Ivor Obeyesekere, 'Alston House', 131 Alston Place, W.A.D. Ramanayake Mawatha, Colombo 2, Ceylon.