Memoranda

MOSQUITO-BORNE HAEMORRHAGIC FEVERS OF SOUTH-EAST ASIA AND THE WESTERN PACIFIC*

INTRODUCTION

During the summer of 1954 an epidemic disease was observed in Manila, characterized by fever, shock, and acute haemorrhage. The clinical and epidemiological characteristics of this outbreak were sufficiently novel for the disease to be named Philippine haemorrhagic fever. There has been considerable discussion since then as to whether the syndrome is in fact that of a previously undescribed disease, and the isolation of the etiological agents has tended to increase this speculation. It is unlikely that the locality of the first appearance of the current epidemics will ever be established, but there is no doubt that mosquito-borne haemorrhagic fever is now a disease of considerable and increasing public health importance in the South-East Asia and Western Pacific Regions of WHO.

Dengue viruses were recovered from patients' sera and from mosquitos during an epidemic in Manila in 1956, but these viruses possessed antigenic components that allowed them to be distinguished serologically from the long-established dengue 1 and dengue 2 viruses. At this stage, it was thought that dengue variants of greatly increased virulence had appeared and that these were responsible for the new disease syndrome. They were named dengue 3 and dengue 4.

With the apparent spread of the disease to Thailand in 1958, not only were two further probable variants of dengue incriminated (tentative types 5 and 6), but also the original dengue 1 and dengue 2 viruses and chikungunya—a serologically unrelated arbovirus, previously known to cause a dengue-like disease in Africa. It will become evident in later sections of this memorandum that the status of chikungunya virus as a causative agent of severe haemorrhagic fever is still far from clear.

The disease syndrome also broadened with succeeding outbreaks, and in some areas only classical dengue symptoms were observed, while in others patients with established disease displayed symptoms ranging from mild pyrexia with or without rash to haemorrhagic fever and death.

In this memorandum all diseases that have chikungunya or dengue viruses as their etiological agents and that occur in the South-East Asia and Western Pacific Regions of WHO are considered.

DISTRIBUTION OF VIRUSES

Philippines

Clinical haemorrhagic fever has been present in recognized epidemic form in Manila since 1954, with peaks of activity every four years. Many other cities and towns on several islands have been involved. Since 1956 there have been isolations of dengue 2, 3 and 4 viruses from patients' sera, and of dengue 3 virus from mosquitos.

Thailand

In Bangkok, clinical haemorrhagic fever was reported to have occurred sporadically in relatively small epidemics for several years before being confirmed by virus isolations in a major epidemic in 1958, and there have been peaks of activity every second year since then. At least four, and possibly six, dengue virus types are present in the Bangkok area, together with chikungunya virus. In general, the disease was restricted to urban areas in the Central Plain and the south-east to south-west coasts until 1964, when significant outbreaks were reported from towns in north and north-east Thailand.

Malaysia [1]

A small number of cases in young adults was observed in Singapore in 1960, and dengue 1 and 2 (or 6 and 5) viruses were isolated. Since then annual

* See page 30 for list of signatories.

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[1] In this memorandum (drafted in 1964) Malaysia is taken to include Singapore, which became an independent State in August 1965.

outbreaks have occurred, and the age-distribution pattern has changed, to affect mainly the younger age-groups; it is now similar to the pattern observed in other countries. Chikungunya virus has not been isolated, but dengue 3 and 4 viruses were recovered between 1961 and 1963.

Haemorrhagic fever was recognized in Penang in 1962, and has been occurring sporadically since then. Dengue 2 virus has been isolated from patients' sera.

Viet-Nam

It appears that clinical haemorrhagic fever occurred in Saigon in 1960, but it was not until the disease spread to the western provinces in 1963 that confirmation by virus isolation was attempted and obtained. Dengue 2 virus has now been recovered from patients' sera and from *Aedes aegypti*, and the presence of dengue 1 is suspected from serological observations. There is also serological evidence of the presence of chikungunya virus, although this virus has not been associated with hospitalized patients.

Laos

A disease clinically resembling haemorrhagic fever was reported in 1962, but the etiological agent is not known.

India

A double-peaked epidemic of disease occurred in Calcutta between July 1963 and March 1964. The first peak was associated with severe heamorrhagic manifestations and was apparently due to dengue virus infections; a dengue 2 virus was isolated. Many isolations of chikungunya virus were made during the second wave of an almost classical dengue-like disease, but some of these appeared to be associated with haemorrhagic manifestations in the patient.

Dengue fever of the classical type has been endemic in parts of India for many years, and until an isolation of dengue 4 was made in Vellore in 1960 the causative viruses were apparently dengue 1 and dengue 2. Dengue 1 was isolated in Calcutta in 1945. Numerous isolations of dengue types 1, 2 and 4 were made in Vellore between 1960 and 1963, but in 1964 these were apparently supplanted by chikungunya virus. No haemorrhagic or shock manifestations have been seen in any of these dengue or chikungunya patients at Vellore.

Chikungunya virus appears to be very widespread in Southern India in 1964, and this seems to be the first occurrence of this agent in India, at least for many years.

Cambodia

Dengue 1 and dengue 4 and chikungunya viruses were isolated from patients in 1961 and 1962, and as yet unidentified group A and B arboviruses have been isolated since then. Human infection appears to be widespread, but no severe cases involving haemorrhage and shock have been reported.

Burma

An epidemic of dengue-like disease with marked arthralgia occurred in Rangoon late in 1963. There have been no attempts to isolate the virus, but fragmentary serological evidence supports the clinical and epidemiological diagnosis of chikungunya virus infection.

Other countries

At the time this memorandum was drafted no definite reports of dengue or chikungunya viruses had been received from Australia, Ceylon, China (Taiwan), Hong-Kong, Indonesia, Japan, Korea and New Zealand.

CLINICAL MANIFESTATIONS

The clinical manifestations of the haemorrhagic disease syndrome have been exceedingly difficult to describe accurately for the following reasons:

(1) As in many infectious diseases, there appears to be a spectrum ranging from mild to fatal infection.

(2) In all regions where the disease has occurred, endemic dengue has been present previously and has occurred concurrently, with clinical manifestations ranging from those of mild undifferentiated disease to those of classical dengue. This and haemorrhagic fever occur in the same season and in the same areas of abundant *Aedes aegypti* population.

(3) In several cities and countries haemorrhagic fever of dengue virus origin has occurred simultaneously with outbreaks of chikungunya virus disease. This disease also presents its own clinical spectrum but frequently is dengue-like in character. Some cases, however, present mild skin haemorrhage; and even severe haemorrhagic manifestations have been reported in rare instances.

(4) Dengue-virus-induced haemorrhagic fever as observed in different countries has presented some significant differences in clinical manifestations.

(5) Viral laboratory confirmation of haemorrhagic fever cases has not been readily available in many areas in past years, and many cases of other diseases have undoubtedly been included in clinical analyses.

The moderately severe and severe types of disease usually present the following picture.

First there is a feverish onset, which may be gradual or sudden but is usually mild for one or two days. This is frequently accompanied by general malaise, sore throat associated with injected pharynx, nausea, vomiting and headache. These become progressively worse, sometimes rapidly, during the second and third days and are followed by a second stage of disease with rather severe abdominal cramps and manifestations of haemorrhage—usually a combination of several of the following: epistaxis, haematemesis, melaena and skin manifestations (including extensive petechiae, or essentially complete subcutaneous or intracutaneous haemorrhage manifested by a non-blanching red-purple skin colour principally on the extremities, face and back, and/or by purpuric spots and large ecchymoses). Cough may then be present. Restlessness or lethargy may ensue, and the temperature begins to fall.

In severe cases the patient, usually on about the fourth or fifth day, very shortly after or simultaneously with the haemorrhagic manifestations, rapidly goes into shock. A few present convulsions. In shock the skin becomes cool and blotchy; the pulse pressure narrows rapidly, and then the blood pressure and pulse become imperceptible. Reports of fatality in shock cases vary from 15% to over 50%. Convalescence, when it occurs, either without the shock phase or following it, is usually very prompt and uncomplicated.

Differences noted in different areas are that the liver is palpable in about half or more of the hospitalized patients in Thailand and Malaysia but is not palpable in those in the Philippines, and that epistaxis is neither frequent nor severe in Thailand, Malaysia and Viet-Nam but is frequent and quite severe in the Philippines.

The proportion of patients reported as presenting the severe signs and symptoms differs markedly in different outbreaks. This appears to be due in large part to the fact that data are collected almost entirely from hospitalized patients and the proportion of patients with mild illnesses hospitalized and diagnosed as suffering from haemorrhagic fever varies greatly. This also affects reported case-fatality rates, which usually vary from 5% to 35%.

At present there appears to be no readily available clinical or laboratory method to differentiate the milder cases of haemorrhagic fever from the spectrum of classical dengue disease. Differentiation has been attempted by the use of the tourniquet test, in the apparently mistaken belief that this is negative in classical dengue. A few petechiae about the ankles and a positive tourniquet test appear to be entirely compatible with classical dengue, and patients without definite signs of more extensive haemorrhage should not be diagnosed as suffering from haemorrhagic fever. Until laboratory confirmation of dengue or chikungunya virus infection is obtained, their illness should be called fever of unknown origin, with or without rash, of unknown etiology. Where the causative virus is determined, the etiological agent should be appended to the descriptive diagnostic terminology. Mild diseases of this nature can be caused by many different commonly recognized viruses of essentially world-wide distribution.

PATHOLOGY, PATHOPHYSIOLOGY AND PATHOGENESIS OF HAEMORRHAGIC FEVER OF DENGUE VIRUS ETIOLOGY

Pathology

Pathological findings in patients who have died, during epidemics of dengue haemorrhagic fever, with a disease characterized by haemorrhage or shock or both have been reported from the Philippines, India and Thailand. No pathognomonic gross or microscopic lesions were found, and specific cellular damage was generally slight. Findings are usually limited to haemorrhages in various organs, evidence of reticulo-endothelial cell reaction, maturation arrest of megakaryocytes or hypocellularity of bone marrow, early degenerative or infiltrative changes in the liver, and interstitial pneumonitis.

Variations in pathological findings have been reported in many cases and from country to country. These may represent differences peculiar to the geographical area or to the outbreak. They may also be explained by inclusion of cases other than of haemorrhagic fever owing to the lack of precise criteria for clinical diagnosis or the lack of etiological identification. These difficulties notwithstanding, most autopsies have shown some evidence of capillary damage, the nature of which has not been discernible with the light microscope. Such damage has resulted in leakage of fluid, plasma and erythrocytes from vessels into interstitial spaces or across serosal surfaces. Varying amounts of fluid are usually found at autopsy in several cavities. The

reticuloendothelial changes have consisted of marked proliferation of lymphocytoid and plasmacytoid cells and a marked increase in phagocytic activities. Maturation arrest of bone marrow megakaryocytes with or without loss of other cellular elements has characterized early stages of the disease. This is followed in many instances by marked cellular hyperplasia. Megakaryocytes are frequently numerous in lung tissue.

Liver changes reported from Thailand and the Philippines have consisted of focal hyaline or acidophilic necrosis of parenchymal and Kupffer cells and infiltration of portal areas of sinusoids by lymphoid cells. In India and the Philippines, paracentral necrosis of liver cells without accompanying inflammatory reaction has been noted.

Mobilization and degranulation of mast cells in the skin during the pre-shock and shock stages have been reported from a series of cases studied in Thailand.

Renal lesions, except for minor haemorrhage, are rare. Pituitary lesions have not been seen. Changes consistent with depletion of adrenal cortical cells are frequently seen. Adrenal cortical haemorrhage is rare. Only occasionally has haemorrhage been considered the possible cause of death. In these cases, bleeding has been either intracerebral or massive gastrointestinal haemorrhage. In most patients in Thailand, pathological abnormalities sufficient to account for death have not been found.

Pathophysiology

Intensive study of hospitalized patients has largely been confined to dengue haemorrhagic fever. Patients hospitalized with chikungunya infections in Thailand have presented a self-limited acute febrile disease with or without positive tourniquet test, spontaneous petechiae, epistaxis, slight liver enlargement, varying degrees of thrombocytopenia, and a normal white cell count or leucopenia. No profound physiological disturbance has been observed in these cases. Recently, chikungunya virus has been isolated from patients dying with a haemorrhagic febrile disease in India. Physiological studies are not available to make possible a comparison of these patients with dengue patients studied in Thailand.

Confirmed severe dengue haemorrhagic fever in the Philippines, Malaysia, Viet-Nam, Thailand and India is characterized by a worsening of the patient's condition after three or more days of fever, the patient presenting cardiovascular collapse and/or gastrointestinal haemorrhage. Studies in Thailand have associated these signs with a shock syndrome characterized by abnormal permeability of the vascular system to fluids and proteins. Such fluid and protein leaks result in decreased plasma volume and haemoconcentration. Patients manifest a mild metabolic acidosis. It has been suggested that the combined effect of these changes is to increase the work of the heart, decrease circulation of blood to tissues, and increase tissue hypoxia. Tissue hypoxia, in turn, heightens metabolic acidosis, producing more capillary damage, allowing greater leakage of fluid and starting some children on an irreversible downward course to death. Death has been observed to be accompanied by marked hyperkaliaemia. Severe gastrointestinal bleeding may accompany the period of shock. Such a complication is common, due to other infections or non-infectious causes.

Thus in addition to the cardiovascular system, other systems are involved.

Haemopoietic. Marked thrombocytopenia has been observed in patients in all countries. This finding has been associated with maturation arrest of megakaryocytes (observed frequently) or increased platelet destruction possibly related to platelet agglutinins demonstrated in a few cases. Possibly both mechanisms operate. In Thailand, a hypocellular and a hypercellular phase have been observed in the bone marrow in some proved dengue virus infections. In severe disease leucocytosis with a shift to the left of the Schilling count has been frequently observed. This is in contrast to classical dengue fever either in haemorrhagic fever endemic areas or in other areas of the world, in which leucopenia occurring late in the illness is the rule.

Gastrointestinal. In addition to haemorrhage in the gastrointestinal tract in severe cases, the organ most frequently showing evidence of abnormality is the liver. In Thailand, liver enlargement is common and early degenerative changes are seen at autopsy. Liver damage may be responsible for the elevations of serum L-aspartate : 2-oxogluturate aminotransferase (serum glutamic oxalacetic transaminase; SGOT) commonly noted even in relatively mild infections, and is probably responsible for the elevations of serum L-alanine : 2-oxoglutarate aminotransferase (serum glutamic pyruvic transaminase; SGPT) and prolonged prothrombin times noted in severe cases.

Pulmonary. Interstitial pneumonitis has been reported from X-ray and autopsy studies. Pleural effusions are found commonly by X-ray or at autopsy and are a manifestation of increased capillary permeability.

Neurological. Marked irritability is common in children with dengue haemorrhagic fever. EEG studies have shown transient abnormalities.

Haemostatic abnormalities. The haemorrhagic manifestations commonly seen in dengue haemorrhagic fever are not completely understood. It seems probable that the positive tourniquet test and spontaneous petechiae are related to capillary damage. In addition, bleeding times are usually prolonged; blood platelets are low; prothrombin times are slightly prolonged (due to abnormalities of factors II, V, VII, IX and X); clot retraction is poor; and blood fibrinogen is slightly reduced. Except in rare cases, measurable haemostatic abnormalities are not below the usual threshold for bleeding established for other haemorrhagic diseases.

Pathogenesis

Broadly speaking, two theories have been invoked to explain the pathogenesis of haemorrhagic fever—that the disease is due to: (1) virulence factors of the virus, or (2) an unusual reaction in the host.

Evidence that a mutant dengue virus (or viruses) is causal is suggested by the apparent spread of haemorrhagic fever to areas previously free from this disease. In India, haemorrhagic fever has been related to type 2 dengue in Calcutta, while in Vellore, in South India, endemic type 2 dengue has been associated only with classical dengue fever.

Antigenic differences, in respect to the magnitude of which there is not complete agreement, have been observed among dengue 1 and 2 viruses isolated in Bangkok. The hypothesis has been put forward that observed antigenic differences may be associated with haemorrhagic and non-haemorrhagic strains within one type. Thus, viruses from severe haemorrhagic fever should be more closely related to TH-36 (dengue 5) and TH-Sman (dengue 6) than to dengue 1 and 2. Further observations are required to test this hypothesis.

Because dengue virus epidemics in other parts of the world have not been accompanied by haemorrhage or shock and because foreign residents of areas where haemorrhagic fever is endemic (largely Caucasians of European origin) have not acquired haemorrhagic fever but, instead, manifest classical dengue fever when infected with local dengue viruses, it has been postulated that host mechanisms may influence the pathogenetic expression of dengue virus infection. The hypothesis that haemorrhagic fever may be some type of hypersensitivity reaction (possibly induced by prior dengue virus infections) is supported by several observations: the unusually high antibody responses noted in many patients with severe haemorrhagic fever; the more rapid disappearance of dengue virus from the blood and tissues of patients with severe disease than from those of patients with mild dengue infection; the frequent epidemiological association of haemorrhagic fever with dengue viruses of multiple types; and the broad group B secondary type complement-fixation antibody response noted in many patients. A second suggestion is that inherited factors more commonly found in Asians than in Caucasians control the system's reaction to dengue viruses. A third suggestion is that nutritional status influences the outcome of disease.

At present, evidence clearly confirming one or another of these hypotheses is lacking.

TREATMENT

The main therapeutic measures that have been described have been for the treatment of patients with haemorrhage (or thrombocytopenia) and shock—especially the latter, which is the principal cause of death in haemorrhagic fever. Most of the patients treated have been severely ill. It has seldom been possible to determine the virus etiology.

The presence of haemorrhage is recognized by the occurrence of frank bleeding, by a fall in the thrombocyte count or by the presence of severe epigastric pain. Blood transfusions are commonly given to counteract haemorrhage, and in one hospital platelet transfusion has recently been adopted instead. Some workers have pointed out that indiscriminate administration of blood to patients without specific indications for transfusion may result in cardiac failure.

The presence of shock is recognized by collapse of the patient, by fall of the blood pressure or narrowing of the pulse pressure, or by various physiological abnormalities. Of the latter, haemoconcentration as determined by the microhaematocrit is considered the most useful guide and should be

estimated every one to two hours during the critical stages of shock.

Different methods of counteracting shock have been described. Most physicians administer fluids such as electrolyte solutions, with or without plasma or blood, the indications being a fall in the blood pressure or narrowing of the pulse pressure. The necessity for withholding fluids during the recovery phase when extravascular fluid returns to the circulation has been stressed.

In addition to fluid replacement, various other procedures have been resorted to, such as the administration of oxygen, hydrocortisone, noradrenaline, aldosterone, phentolamine sedatives and antibiotics. Indications for such supplementary measures are not well defined, and there is no general agreement regarding their use.

The results obtained by different physicians are difficult to evaluate, as the numbers of cases treated have usually been small and there have been no controlled trials. A number of clinicians feel that they cannot carry out controlled trials when this would mean depriving some patients of what they consider useful. However, many of the methods proposed have been so seriously challenged that it appears entirely justifiable to conduct adequately controlled clinical trials.

ETIOLOGICAL DIAGNOSIS

A specific etiological diagnosis can be made only in a laboratory capable of carrying out virological tests.

Dengue virus serology

Serological methods are very commonly employed, but they can seldom do more than determine that the illness is associated with a rising titre to any of a number of agents of the arbovirus group B, which includes the dengue viruses. The serological diagnosis of virus infection is an inference made on additional and essential clinical and epidemiological information. It is seldom possible to determine which type of known dengue virus is involved when doing serology in an endemic dengue area, since a majority of patients will have had previous infections with immunologically related viruses, and, owing to an anamnestic response, the highest antibody titre may well be to one of the agents responsible for a previous infection.

Three types of serological test are in common use. Each has its advantages and disadvantages, and at least two types of test are employed in some laboratories to clarify the diagnosis where doubt exists.

Complement-fixation (CF) and haemagglutination-inhibition (HI) tests. These two tests are the most rapid and most commonly used. Antibodies of these two types form at about the same time in dengue virus infections, so interpretations are made in about the same way. In laboratories without previous virological experience, the CF test is simple to perform, but those having the necessary skills and equipment may prefer the HI test. The antigens most commonly used for both tests are difficult and somewhat dangerous to prepare, so most laboratories should depend on supplies of these and control antisera from elsewhere. The antigens most frequently used are of the acetone-ether or sucrose-acetone types. These are infectious when freshly prepared or stored frozen or lyophilized. *Therefore, unless specially inactivated, antigens representing viruses not known to be present in an area should not be imported or employed except under unusual circumstances and with suitable government permits.*

The following suggested interpretation is applicable to dengue infections:

When interpreting results it is necessary to consider both the time of collection in respect to the illness and the interval between specimens. In general, a fourfold rise is considered significant. However, if both specimens are collected after the fifth day from the onset, one may expect rather high titres of antibody without demonstration of a significant increase.

The following criteria are recommended:

Positive: if during the course of clinical disease HI and/or CF antibody to any dengue virus (or possibly another group B arbovirus such as Murray Valley encephalitis virus) exhibits a fourfold or greater increase in titre, or if both specimens are collected after the fifth day from onset and show rather high titre to dengue viruses (e.g., in one Bankgok laboratory, a HI titre $\geq 1:2560$, or CF titre $\geq 1:64$) without demonstration of a significant rise.

Inconclusive: if both acute and convalescent sera show some antibody (e.g., HI titre $\leq 1:1280$, or CF titre $\leq 1:32$) without demonstration of an increase.

Negative: if HI and CF antibody are not demonstrable throughout the course of the illness.

The inconclusive results and the positive results without titre rise are assumptions based on the knowledge that these two antibodies decrease in titre quite rapidly following their peak rise. Such

titres would not be expected from previous infections, unless very recent (very high positive) or not quite so recent (low inconclusive). The titres shown as examples may not apply to tests performed in other laboratories and with other types of antigen or with modifications of the test. Local experience must be gained to interpret anything but a significant titre rise. A rather rapid titre fall between early and late convalescent sera has been considered diagnostic by some and inconclusive by others.

A modification of the HI test has recently been described—the " sensitized erythrocyte agglutination " (SEA) test. This also may be used for diagnostic serology. Advantages claimed for this test are reduced cross-reaction with other group B arboviruses yet such extensive crossing among the dengue viruses that type 1 dengue antigen may be used alone and kaolin extraction of sera may be omitted. Among the disadvantages are the large amounts of antigen required and the fact that antigen preparations cannot be stored in excess of a week.

Neutralization test (NT). This test is usually performed in mice, mixtures of undiluted serum and various virus dilutions being inoculated intracerebrally in susceptible mice. Some dengue viruses have been adapted to kill weanling mice; other to kill sucklings only. The mice are observed for a reasonable period of time and calculations made of the neutralization index. A 10-fold or greater change in neutralization index between an acute and a convalescent phase serum is usually required for demonstration of a titre rise. The optimum time for incubating serum-virus mixtures before inoculation has not been determined, but the avidity of some sera has been known to be very great.

Tissue culture methods for performing neutralization tests have been developed very recently. Their usefulness is rapidly increasing with improvements in techniques and experience. None will be described in detail.

Challenge interference is performed on monolayers with any of several continuous cell lines or primary cells of monkey origin. Frank cytopathogenic effect (CPE) is seldom observed with dengue virus. The presence or absence of free infective virus is detected by inoculating the cell sheet with a mixture of serum and virus; incubation is carried out for a few days; then a virus capable of producing complete CPE in the non-dengue-infected cells is added. If CPE occurs, the dengue virus is shown to have been inactivated by the serum, while if no CPE occurs the serum did not contain adequate antibody. This test can be used quantitatively by employing a constant amount of virus and serial serum dilutions.

Several methods of plaquing dengue viruses have been developed recently, and various methods of plaque neutralization may be used. These have had very limited use so far, but they may well become the method of choice in time.

Chikungunya virus serology

Serological tests for chikungunya virus infections may be conducted in the same manner as for dengue virus but are less complicated in several ways: (1) results of CF, HI and NT are much more specific among group A than among group B arboviruses; (2) only one serological type of chikungunya virus is recognized; (3) antibodies to this virus rise less rapidly than do those to dengue viruses, so there is a better opportunity to detect a significant rise in titre between a late acute phase serum and one taken during convalescence. This latter, however, presents one difficulty in the use of the CF test, for CF antibody rises so slowly that a serum taken on the 10th or even the 15th day may not yet contain detectable antibody. Therefore, the HI or NT method is usually preferred. NT can be readily performed in tissue culture by using a tissue culture monolayer with direct reading of clear-cut CPE. Hamster kidney tissue culture is highly susceptible.

Where both chikungunya and dengue viruses are active it is important to conduct tests for both viruses on all specimens, as more or less simultaneous infections with both viruses have been observed frequently.

Virus isolation and identification

Dengue viruses. Isolation of dengue viruses, whether from man or mosquitos, is a most difficult procedure. Most data available suggest that the strains producing the haemorrhagic disease syndromes in South-East Asia are particularly difficult to isolate. Isolation from mild and classical dengue-like cases is much more likely to succeed. Reasons for this are advanced, based on two hypotheses: (1) the strains of high pathogenicity for man have low neurovirulence for the mouse; (2) persons developing severe disease develop antibody more rapidly, and virus is likely to be neutralized in even the early acute phase serum.

Isolations are much more likely to be made from serum drawn as soon as possible after onset, and the chances of isolation decrease with time. Isola-

tions appear to be made more frequently from diluted serum, usually in a range between 1:4 and 1:40 but occasionally are successful only at an even higher dilution. Neutralizing antibodies are frequently readily detectable in the serum from which the isolation is made. Isolations from tissues or blood at autopsy have been extremely rare.

The most commonly used method involves mice. Very young suckling mice are injected intracerebrally and intraperitoneally. Serial blind brain passage is frequently necessary before disease is observed. These passages are made at about 10-day intervals. Information on whether blind passage is likely to succeed is obtainable by holding a litter of inoculated mice for 21-28 days, then challenging intracerebrally with about 100 to 1000 LD_{50} of any dengue virus which has been well adapted to kill weanling mice. If one-third or more survive this challenge, that is evidence of the presence of an infective amount of virus in the original inoculum. If none survive, there was probably little or no virus in the specimen tested, and serial blind passage is unlikely to be productive.

When mice develop signs of illness (frequently paralysis in either primary or subsequent passage) then serial passage is maintained until all inoculated mice die with regularity and within a few days of one another. At this time (varying from about three to 10 passages) a complement-fixing antigen can frequently be prepared from a pool of brains and tested against an appropriate series of type-specific identifying sera, or suckling mouse neutralization tests can be performed against a similar battery of sera. A hyperimmune serum should also be prepared against the new agent and tests made with it by CF or NT against a battery of known dengue virus antigens. Identification actually consists in typing.

There is still uncertainty as to how many types of dengue virus exist. There are at least four, and there is highly suggestive evidence of six and quite possibly more. All dengue viruses appear to cross-react to some degree by every test procedure, unless the test is one of very low sensitivity. This crossing places them in the dengue subgroup. Dengue viruses appear to vary greatly in antigenic composition. Each has at least one major antigenic component and several minor ones, representing any or each of the other types. Frequently a virus has at least two antigenic components of essentially equal magnitude, and to know by which of these components it should be named becomes a problem. Testing both the antigen and an antiserum made from it may help to reveal which component is the major one. Other viruses appear to contain three or more components of essentially equal magnitude, and the present typing system does not provide for these.

Typing of dengue viruses is at present in an early, crude stage, inadequate for good epidemiological study of cases. As yet it has not been possible to distinguish between the strains or types that produce haemorrhagic fever and those that produce classical dengue. Crudely classifying all strains as dengue 1, 2, 3 or 4, regardless of their many obvious differences, has certainly not resulted in such distinction, since viruses that most closely resemble each of these types have been found in different parts of the world producing dengue and also in South-East Asia in association with epidemics of haemorrhagic fever. However, other arbovirus diseases caused by closely related agents have been satisfactorily distinguished by antigenic typing, so it is expected that this group will eventually be adequately typed on an antigenic basis. A notable recent example of success in another subgroup of group B is the differentiation of three types of encephalitis and of two types of haemorrhagic fever viruses, caused by five members of the Russian-spring-summer–louping-ill virus group, the haemorrhagic fevers of which are caused by Omsk virus and by Kyasanur Forest virus. These agents are as closely related as are those of the dengue subgroup, and years of work were required to develop dependable methods to distinguish one from the other in the laboratory.

Another typing method—that is of low sensitivity, but is rapid and requires only one mouse brain of early passage—is the micro gel-precipitin method. Undiluted crude mouse-brain suspension is placed in the central well; high-titre typing antisera are placed in equidistant wells surrounding it. The method appears to be highly specific, and might allow more critical analysis of the antigenic components of dengue viruses.

Newer tissue culture developments offer great promise for dengue virus isolation and typing. Certain tissue cultures of monkey kidney origin, as described in the discussion of serological methods above, are susceptible to dengue infection, though not necessarily to CPE. The infected cells resist CPE when challenged with a virulent CPE-producing virus at a later date. The sensitivity of this method appears to be roughly parallel to that of mouse inoculation. After a varying number of passages to establish a reasonable titre, neutralization tests with typing sera can be carried out by the same indirect method. This method saves one from the many

problems encountered in blind passages and prolonged holding of suckling mice, most of which carry several latent viruses which may be activated by an additional infection or trauma.

Direct plaquing methods in tissue culture are relatively untried but offer promise for isolation and typing.

Production of suitable typing sera for dengue is one of the current problems needing solution. Human sera collected from persons recovered from dengue virus infections who have not had a previous group B arbovirus infection have proved to be most type-specific. Such sera, however, are not readily available for all types and are seldom available in adequate quantities. Most laboratory animals require multiple injections of virus to gain antibody titres of adequate level for reliable work; such animal sera suffer from a lack of type specificity. Monkeys without preceding group B antibody develop adequate levels of neutralizing antibodies three to four months after a single injection of virus, but their sera can seldom be employed for CF or HI unless repeated injections are given. Guinea-pigs and hamsters require more study as possible sources of typing sera.

Absorption techniques can be employed for obtaining greater specificity of typing sera, but these are time-consuming and expensive and have been but partially investigated for dengue viruses.

One major problem, still unsolved and related directly to dengue virus typing, is that of the probable selection of a minor virus population from the original inoculum through serial passage in a host tissue of extremely low susceptibility, such as the mouse, and possibly many tissue culture cells. Apparently only a few virus particles are capable of successful invasion of the brain cells of the mouse. These selected particles are gradually increased in number through serial passages. It is doubtful whether these retain the full antigenic spectrum of the major virus population reproducing in the original host. Thus, employing these for a prototype may be very misleading. Direct plaquing techniques may avoid this, but this again will depend to a great degree on the plaquing efficiency of the culture for the predominant virus population. The problem is obviously very different from that of most arboviruses, which go readily in the first passage in mice. Even these, however, have been shown to undergo marked selection in one or a very few passages. Dengue viruses of types 1 and 2 have been shown to undergo marked attenuation for man after only a few mouse passages and can then be used for attenuated live virus vaccines without production of more than very minor illness or rash. This is mentioned as an example of selection by passage.

Chikungunya virus. The isolation of chikungunya virus is usually a relatively simple procedure with most strains in suckling mice, and blind passage or extended serial passage is not required. Some difficulty has, however, been encountered with Cambodian strains. Identification can frequently be made with brains from the first passage. One unusual difficulty is encountered in serial passage; a 10% mouse-brain suspension may fail to kill any mice because of " auto-interference ". This is avoided if a 10^{-2} or a 10^{-3} dilution is routinely used for all passages.

Isolation may also be made in certain types of tissues culture by observation of CPE or by plaquing.

In contrast to what is true for practically all other group A arboviruses, the embryonated egg is not susceptible to this virus.

ECOLOGY OF THE VECTOR

It has been established that *Aedes aegypti* is the primary vector species of dengue and chikungunya viruses in areas where haemorrhagic fever has been reported. This conclusion is based upon: (1) epidemiological observations, including distribution of *Aedes aegypti* in space and time in relation to incidence and distribution of cases, (2) numerous isolations of dengue and chikungunya viruses from naturally infected *A. aegypti* mosquitos, and (3) limited experimental transmission studies. This does not preclude the likelihood that *Aedes albopictus* is a vector of dengue and chikungunya viruses, but probably not associated with haemorrhagic fever.

Aedes aegypti is generally considered to have been introduced and established in South-East Asia some time before the year 1900. It is now widespread in coastal urban areas of South-East Asia and India, and has spread to inland cities and towns following the main lines of communication.

Aedes aegypti is highly anthropophilic and feeds predominantly indoors during the daylight hours. It breeds almost exclusively in containers in and around human dwellings. It is not known to have adapted to a sylvan environment in these areas, although a few scattered observations have been made of tree-hole and rock-hole breeding.

No vertebrate host other than man has been demonstrated conclusively for the dengue viruses. Serological studies in Malaysia, however, have shown good presumptive evidence of widespread dengue infection in wild monkeys in forest areas away from normal human activity. The suggestion that the dengue viruses may be zoonoses should be given serious consideration. *Aedes albopictus*, which is widespread in most urban, rural and jungle areas, is considered the most likely native vector of endemic and possible jungle dengue viruses.

There is evidence to suggest that chikungunya virus may have a zoonotic cycle in Thailand. Chikungunya antibodies have been demonstrated in several large domestic animals, and the virus has been recovered from *Culex tritaeniorhynchus* and *Culex gelidus* mosquitos collected on a horse farm.[1] This suggests a wild cycle involving *Culex* mosquitos and vertebrates other than man, in addition to the urban *A. aegypti*–man cycle.

EPIDEMIOLOGY

Since they were first described from the Philippines in 1956, haemorrhagic fever outbreaks have been reported from cities, towns and villages in the South-East Asia and Western Pacific Regions. The disease has shown a tendency to be recurrent and to spread to adjacent areas.

Epidemic haemorrhagic fever is a disease of the rainy season and of the period of *Aedes aegypti* abundance. However, in highly endemic areas such as Bangkok or in tropical areas without marked seasonal rainfall, cases may occur in every month of the year.

Disease has appeared first in port cities and railroad centres. Transmission of dengue and chikungunya viruses from house to house and from city to city is probably brought about by the movement of infected persons during the incubation period. Many of these may have only mild illnesses. As long as houses or cities are infested with *Aedes aegypti*, epidemics may occur through the introduction of virus. Ships of all sizes provide an opportunity for mosquito infestation and breeding. With a sufficient number of persons on board, man-to-mosquito-to-man transmission may occur, circulating viruses over relatively long periods of time. The transport of infected *Aedes aegypti* or infected human beings by aeroplanes provides a possible means of spread of viruses from country to country.

In Thailand and India, chikungunya in addition to dengue viruses have been isolated from patients hospitalized with a diagnosis of haemorrhagic fever. Studies in Bangkok have shown that chikungunya virus is transmitted during the rainy season by *Aedes aegypti*, with an epidemic curve which may not completely coincide with that of dengue virus transmission. Up to 15% of hospitalized haemorrhagic fever patients in Bangkok have chikungunya disease. Close examination of the clinical course of these illnesses, however, suggests that they do not progress to the severe or fatal haemorrhagic fever syndrome. Disease caused by chikungunya virus is difficult to differentiate from mild dengue infection. In Calcutta, serological and retrospective epidemiological studies have shown that an outbreak of dengue disease occurred during the months July to November 1963 and an outbreak of chikungunya between September and December 1963. The former disease was thought to be more associated with haemorrhagic cases and deaths and the latter with a dengue-like syndrome which had extremely high attack rates. In 1964, chikungunya became epidemic throughout South India, haemorrhagic complications and deaths being reported in a few cases.

Multiple dengue virus types have been reported from outbreaks in the Philippines (types 2, 3 and 4) and Thailand (types 1, 2, 3, 4, TH-36, TH-Sman) and from dengue-like disease in South India (types 1, 2 and 4) and in Cambodia (types 1 and 4). On the other hand, from some outbreaks of haemorrhagic fever only one dengue virus type has been isolated. Thus, in Penang, Viet-Nam and Calcutta, only dengue type 2 has been identified. Since intensive prospective studies of many cases have not been possible in each area, it is too early to conclude that the presence of other types has been excluded.

Severe haemorrhagic fever in the Philippines, Viet-Nam, Malaysia and Thailand has been a disease of children. In large outbreaks there is no sex predilection. In Thailand and Viet-Nam, maximum age-specific hospitalization rates occur from 3 to 5 years of age, while in several other countries the modal age is slightly higher. Recently, a severe syndrome resembling haemorrhagic fever has been recognized in young adults in Thailand. In the Calcutta outbreak, children were more commonly affected with severe disease than adults, but a substantial number of severe illnesses and deaths

[1] *Culex tritaeniorhynchus* and *C. gelidus* have not been tested in the laboratory for transmission of chikungunya virus. *Culex pipiens fatigans* (= *C. pipiens quinquefasciatus*) failed to transmit chikungunya virus experimentally.

occurred in adults. Whether the relative lack of cases in adults in most countries is related to immunity or to age-related host factors is not known.

Since a number of foreigners with quite different ethnic backgrounds and immunological and dietary status reside in large urban centres in the haemorrhagic fever endemic areas, it has been of considerable interest to observe the type of dengue disease acquired by this group. To date, no laboratory-documented dengue haemorrhagic fever (shock or severe haemorrhage) has been recognized in a Caucasian of European descent. In Thailand, over 150 dengue virus infections are on record, including 70 infections with a type 1 dengue virus occurring in an outbreak in a small town in which there were concurrent cases of haemorrhagic fever in Thai and Chinese children. In each instance in a foreign Caucasian only the spectrum of signs and symptoms characteristic of classical dengue fever has been observed. It is not known whether sufficient numbers of dengue infections have occurred in non-indigenous Caucasians to provide statistically significant evidence of resistance to haemorrhagic fever in this group. None the less, the typical dengue fever acquired by Caucasians shows that some dengue viruses in the South-East Asia and Western Pacific Regions still retain their ability to cause this form of the disease.

CONTROL MEASURES

Other than the control of the vector, *Aedes aegypti*, no adequate preventive or control measures are available to deal with epidemics of dengue or chikungunya in urban areas. No vaccine has as yet been developed that is considered suitable for use in the areas of the outbreaks.

Small-scale pilot *Aedes aegypti* control projects in Bangkok, using DDT as a larvicide and adulticide, have met with some success. However, susceptibility tests from several countries indicate that the gene for DDT-resistance is now widespread throughout the South-East Asia and Western Pacific Regions. Where tests have been performed in Thailand, both larval and adult *Aedes aegypti* were demonstrated to be highly resistant to DDT, and in may soon be necessary to consider an alternative insecticide. Before the use of any other insecticide is considered, it is essential that adequate base-line susceptibility data be obtained.

Public co-operation in preventing the breeding of *Aedes aegypti* in domestic water containers is most important; for this, a carefully planned, professionally administered control programme, integrating all means of control, is necessary. This should include public health education, public health engineering, larviciding and adulticiding. When an emergency exists in the face of severe and even repeated epidemics, such as in Bangkok and several cities in other countries, an immediate control programme might well be instituted. At least, strong efforts should be made to reduce the level of the vector population low enough to interrupt transmission of the disease. But it is essential that such a programme have competent professional direction, to take advantage of information and experience already available from other programmes and apply it to the local situation; otherwise, efforts and funds will certainly be uselessly dissipated. For long-range programmes, beyond immediate measures, careful planning and additional surveys and research are desirable before large-scale city-wide or country-wide campaigns are undertaken. This need has been shown by recent interruption of an *Aedes aegypti* eradication campaign in the Carribean owing to widespread development of insecticide-resistance. A number of new larvicides of low mammalian toxicity, suitable for use in domestic water storage containers, are available for trials. A number of chlorinated hydrocarbon and organophosphorus insecticides are also currently available for immediate use in a control campaign, should susceptibility tests demonstrate that there is no resistance to them. Any insecticide, either a larvicide or a residual adulticide, should be characterized by a long period of persistence, or the labour costs involved in frequent reapplication may preclude its use.

After careful study of the biology and ecology of the vector, future possibilities of control include the sterile-male technique or such genetic means as the introduction of incompatible or lethal genes or control by parasites or predators.

A fundamental method of reducing the vector population remains the provision of adequate piped water supplies into the home, reducing dependence on storage containers. Along with this, general sanitation should exclude secondary breeding in tin cans, tyres, etc. Until such an ideal situation exists, however, reliance must be placed on insecticide treatment.

INTERNATIONAL CO-OPERATION

Although intensive studies have been carried out in recent years in countries where haemorrhagic

fevers have occurred, complete knowledge about the geographical areas affected is not yet available It is consequently necessary to determine the real extent of the problem. Suggestive evidence of the distribution of dengue and chikungunya viruses should be obtained by carrying out serological surveys in different areas of the countries in South-East Asia and the Western Pacific.

Blood samples should be obtained by random sampling from children and young adults from several areas in each country. Paired samples of sera should also be obtained from suspicious cases in all hospitals in these areas. The presence or absence of antibodies against dengue and chikungunya viruses in these samples should be determined.

As long as non-infectious antigens are not available, sera could be sent to WHO Reference Laboratories and other specialized laboratories for study. These laboratories, after carrying out the necessary tests, will compile the information obtained and send it to some appropriate international body or centre for distribution among other laboratories and health authorities interested in this problem.

If and when non-infectious antigens become available, they may be distributed among public health and hospital laboratories, which should perform relatively simple serological tests such as CF and HI.

In those laboratories with suitable facilities attempts should be made to isolate viruses from patients and mosquitos.

The setting up of such a programme would make it possible to collect valuable information on the distribution of these viruses throughout South-East Asia and the Western Pacific.

It would be in the interest of all countries concerned to make available for general dissemination regular and current reports weekly on the occurrence of cases and outbreaks of haemorrhagic fevers. Epidemics of dengue-like disease should also be reported monthly. If possible, the specific etiology of the disease (dengue type, chikungunya, etc.) should be mentioned in this report. It is specially important that the presence of cases of haemorrhagic fever be reported from cities or towns adjacent to international ports or airports.

Under the International Sanitary Regulations, countries have an obligation to keep their ports and the area within the perimeter of airports free from *Aedes aegypti*. As *Aedes aegypti* is the primary urban vector of dengue and chikungunya viruses, it is of the utmost importance that all countries fulfil this obligation to prevent the implantation in these areas of a dangerous dengue virus strain brought in during the incubation period by passengers coming from affected areas. It would be desirable to extend this control to large adjacent towns and cities whenever feasible, because travellers do not restrict themselves to the areas immediately surrounding ports and airports.

The International Sanitary Regulations provide for the destruction of insects in aircraft if there is reason to suspect the importation of insect vectors. It should be remembered, however, that the carriage of infected mosquitos is probably less important than the carriage of virus by an infected person.

A good precautionary measure would be the adoption, in appropriate countries, of the following practice: incoming passengers from areas known to have mosquito-borne haemorrhagic fevers should be required to report to the Health Department any febrile disease developing at an interval up to 10 days from their departure from the affected areas; this would give time, in case of a haemorrhagic fever diagnosis, to proceed to the destruction of mosquitos in buildings visited by the recently arrived persons during the incubation period of the disease.

A prophylactic vaccine is unfortunately not available at the present time. Efforts are being made to develop such a vaccine, but it cannot be anticipated that it will be available in the near future.

SUGGESTIONS FOR FURTHER STUDIES

1. Further research is desirable on improved methods for isolating dengue viruses—methods that are rapid, sensitive and not likely to modify the character of the original virus population found in the invertebrate or vertebrate host. Plaque isolation in tissue culture deserves particular attention. Methods to dissociate virus from antibody in human specimens should be explored.

2. Improved methods of antigenic analysis for the typing of dengue virus deserve increased effort.

3. When adequate typing is established, it is exceedingly important to determine whether it is possible to differentiate viruses causing the severe haemorrhagic syndrome from those causing classical dengue.

4. Further study needs to be directed at determining whether host factors, such as immunological sensitization or dietary or genetic factors, play

essential roles in determining susceptibility to the haemorrhagic syndrome.

5. Studies are needed to localize the tissues and organs in which virus multiplies in man, to assist in explaining pathogenesis and to indicate appropriate sources for virus isolation at autopsy.

6. Studies must be extended on the physiology of the shock and pre-shock stages to enable a more rational approach to the prophylaxis and therapy of this manifestation of the disease. This will require close co-operation between physiologists and clinical pharmacologists in carrying out well designed, controlled tests of therapeutic methods, and the immediate initiation of such tests should be actively encouraged.

7. Classical dengue in areas free from haemorrhagic fever should be studied carefully by haematologists to determine similarities and dissimilarities related to haemopoietic tissues, platelets and haemostasis. Capillary fragility tests and capillary biopsies may prove enlightening.

8. *Aedes albopictus* should be tested in the laboratory in parallel with *Aedes aegypti* for quantitative, comparative transmission efficiency, use being made of dengue viruses of several antigenic types isolated from true haemorrhagic fever cases.

9. *Culex tritaeniorhynchus* and *Culex gelidus* should be tested for ability to transmit chikungunya virus.

10. A variety of domestic and wild mammals and birds as well as amphibia and reptiles should be inoculated with chikungunya virus and tested for viraemia and antibody response.

11. Continued studies are required in search of a zoonotic forest reservoir of dengue viruses. If viruses in a sylvan cycle are recovered, they should be compared with those of classical dengue and of haemorrhagic fever.

12. Inactivated polyvalent dengue virus vaccines require further exploration, particularly from tissue-culture sources.

13. The establishment of effective and long-lasting vector control measures in South-East Asia and the Western Pacific should be preceded and accompanied by extensive research on *Aedes aegypti* and related species. This research should include confirmation of vector ability, vector distribution, bionomics, ecology and insecticide-susceptibility.

14. Attempts should be made to utilize the high titres of dengue and chikungunya virus antigen present in acute phase sera in an immediate *in vitro* diagnostic test.

15. Suitable diagnostic criteria and a classification and nomenclature based on symptoms, physical examinations and clinical laboratory findings (including haematology and blood chemistry), should be developed that would be applicable to all variations of the diseases caused by dengue and chikungunya viruses, as seen in the different countries where these are etiological agents of haemorrhagic fever. A suggested system is presented in the annex to this memorandum.

16. There is a need for continued—and, indeed, increased—co-operation among the various research and clinical groups and individuals engaged in work on haemorrhagic fever and within and between cities and countries. Communication of an informal nature and through organized conferences, small and large, can be expected to contribute significantly towards these goals.

Annex

SUGGESTED DEFINITION AND NOMENCLATURE FOR DISEASES SUSPECTED TO BE OF DENGUE OR CHIKUNGUNYA VIRUS ETIOLOGY

It must be recalled that mosquito-borne fevers are multiple in etiology. In addition, many fevers of a mild and relatively undifferentiated nature are caused by enteroviruses and by many other infectious agents. The physician seeing a patient with such an illness during an epidemic which is believed to be caused by an arbovirus can seldom differentiate clinically in any individual patient in respect of etiology, and should be content to call the ailment a fever of unknown origin or an undifferentiated fever. The presence or absence of a rash adds little of diagnostic significance. When the etiology is established and the clinical syndrome is undifferentiated, diagnosis should still be one of undifferentiated fever of the specified etiology, e.g., due to dengue virus type 3 or due to chikungunya virus.

A reasonably well established clinical syndrome of dengue-like disease has been recognized and fairly well accepted for many years. Certain cases in some countries and in certain age-groups (usually adults) fit easily into this category. The etiological diagnosis, if known, should be added; e.g., dengue fever syndrome due to chikungunya virus.

If the disease is associated with relatively severe haemorrhagic manifestations and/or shock and is in other ways compatible with the more severe grades of what has been called Philippine, Thai or Singapore haemorrhagic fever, the term " haemorrhagic fever "[1] would be indicated. Again the etiology should be added, if it is known.

On the basis of the above, the following classification and nomenclature is suggested:

1. *Undifferentiated fever :*
   (i) *etiology unknown*
   (ii) *etiology stated*

2. *Dengue fever syndrome* (this should include febrile disease characterized by myalgia and/or arthralgia and leucopenia, with or without rash or lymphadenopathy, but including many of the following: biphasic fever, severe headache, pain on moving the eyes, positive tourniquet test [2] and a few spontaneous petechiae):
   (i) *etiology unknown*
   (ii) *etiology stated*

3. *Haemorrhagic fever* [3] (This includes fever, usually without prominent myalgia or arthralgia, positive tourniquet test,[2] leucopenia or rash present or absent, but usually with several of the following: extensive spontaneous petechiae, purpura, echymoses, epistaxis, haematemesis, melaena, thrombocytopenia, prolonged bleeding time and maturation arrest of megakaryocytes):

   A. *Without shock :*
      (i) *etiology unknown*
      (ii) *etiology stated*

   B. *With shock.* (The pulse pressure is 20 mm Hg or less, or systolic and diastolic pressures unobtainable, with collapse of the patient. Shock may occur without the haemorrhagic manifestations described above but with most of the following associated with serious disturbance of the haemostatic mechanism as essential criteria if haemorrhagic fever has been diagnosed: positive tourniquet test, thrombocytopenia, prolonged bleeding time and maturation arrest of the megakaryocytes):
      (i) *etiology unknown*
      (ii) *etiology stated*

[1] In areas where tick-borne or mite-borne haemorrhagic fevers also exist, it might be wise to use the longer title " mosquito-borne haemorrhagic fever ", provided that the other types can be reasonably well ruled out on epidemiological grounds.

[2] The tourniquet test should be employed in a standardized manner (e.g., the Rumpel-Leede test) It should be done with a blood-pressure cuff and not with a tourniquet.

[3] To be considered in differential diagnosis are: thrombocytopenic purpura, haemophilias of genetic origin, meningococcaemia, scarlet fever, haemorrhagic measles, rubella, enterovirus infections with rash, rickettsial spotted fevers such as scrub typhus, leptospirosis, etc.

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C. Y. CHOW, Regional Entomologist, WHO Regional Office for the Western Pacific, Manila, Philippines
NORMAN G. GRATZ, Scientist-Entomologist, Vector Control, World Health Organization, Geneva, Switzerland
SCOTT B. HALSTEAD, Chief, Virus Department, US Army-SEATO Medical Research Laboratory, Bankgok, Thailand (present address: Yale Arbovirus Research Unit, New Haven, Conn., USA)
W. M. HAMMON, Professor and Head, Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa., USA
LIM KOK ANN, Department of Bacteriology, University of Singapore, Singapore
IAN D. MARSHALL, Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, Australia
J. A. MONTOYA, Medical Officer, WHO Regional Office for South-East Asia, New Delhi, India
ALBERT RUDNICK, International Center for Medical Research and Training, G. W. Hooper Foundation, University of California Medical Center, San Francisco, Calif., USA
A. C. SÁENZ, Medical Officer, Virus Diseases, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland
PRAKORB TUCHINDA, Department of Pediatrics, Faculty of Medicine and Siriraj Hospital, University of Medical Sciences, Bangkok, Thailand

RÉSUMÉ

La fièvre hémorragique transmise par les moustiques fit son apparition à Manille au cours de l'été 1954. Ses aspects épidémiologiques et son tableau clinique, caractérisé par la fièvre, le choc et des hémorragies aiguës, la firent considérer à l'époque comme une maladie nouvelle, qui prend actuellement une importance considérable. La fièvre hémorragique a été attribuée successivement à des virus de la dengue distincts des types 1 et 2 connus depuis longtemps, à ces mêmes virus des types 1 et 2 et au virus chikungunya dont la responsabilité n'est pas encore clairement établie.

Les modalités épidémiques de l'affection sont très variables suivant les pays. Depuis son apparition à Manille en 1954, elle a présenté aux Philippines un maximum d'activité tous les quatre ans; les virus de la dengue des types 2, 3 et 4 ont été isolés à partir de sérums de malades et le virus du type 3 chez des moustiques. En Thaïlande, de petites épidémies ont sévi pendant plusieurs années avant que des virus soient isolés au cours d'une épidémie plus importante en 1958. Les maximums épidémiques se reproduisent tous les deux ans. On trouve à Bangkok au moins quatre et peut-être six types de virus de la dengue ainsi que le virus chikungunya. En 1964, la maladie a gagné les villes du nord et du nord-est de la Thaïlande jusque-là indemnes. A Singapour, des virus de la dengue des types 1 et 2 ont été isolés en 1960 d'un petit nombre de cas; depuis, des poussées annuelles affectent surtout les groupes d'âge les plus jeunes. Les virus de la dengue des types 3 et 4 ont été isolés entre 1961 et 1963 et le type 2 a provoqué quelques cas sporadiques à Penang depuis 1962. Au Viet-Nam, Saïgon a été touché en 1960 mais des virus n'ont été isolés qu'en 1963. Le virus de la dengue du type 2 a été identifié chez des malades et chez *Aedes aegypti*; le type 1 et le virus chikungunya seraient aussi responsables de certains cas d'après des observations sérologiques. Au Laos, une maladie cliniquement semblable à la fièvre hémorragique existe depuis 1962 sans qu'aucun virus ait été isolé. En Inde, deux poussées épidémiques ont été observées à Calcutta depuis juillet 1963. La première, caractérisée par des manifestations hémorragiques graves, était apparemment due à des virus de la dengue; au cours de la deuxième, les cas ressemblaient à la dengue classique, et le virus chikungunya fut isolé à de nombreuses reprises. La dengue classique, endémique en Inde, semble avoir été causée par les virus des types 1 et 2 jusqu'en 1960 où fut isolé le type 4. Depuis 1964, à Vellore, il apparaît que le virus chikungunya a remplacé les virus de la dengue fréquemment isolés auparavant. Au Cambodge, l'infection par les virus de la dengue des types 1 et 4, le virus chikungunya et des arbovirus non identifiés appartenant aux groupes A et B est largement répandue mais ne s'accompagne ni d'hémorragie ni de choc. En Birmanie, une épidémie d'une affection rappelant la dengue est apparue à Rangoon à la fin de 1963. Aucun virus n'a été isolé mais la sérologie, la clinique et l'épidémiologie plaident en faveur d'une infection à virus chikungunya.

Il est particulièrement difficile de décrire avec précision le tableau clinique de la fièvre hémorragique: l'affection est tantôt bénigne, tantôt fatale avec tous les degrés de gravité intermédiaires; elle coexiste avec la dengue classique, elle-même d'allure très variable; les deux maladies se manifestent à la même saison, et sont liées à la présence en grand nombre de *A. aegypti*; on note fréquemment l'apparition concomitante d'épidémies d'infections à virus chikungunya, qui peuvent être également responsables d'hémorragies cutanées discrètes, ou parfois graves.

Les formes graves et modérées débutent habituellement par une fièvre bénigne durant 1-2 jours avec symptômes généraux notables. Après une aggravation progressive, parfois rapide, le 2e-3e jour, apparaît la seconde période avec crampes abdominales et manifestations hémorragiques diversement associées: épistaxis, hématémèse, mélaena, pétéchies, suffusions sanguines sous-cutanées. L'agitation ou la torpeur lui succèdent parfois, avec baisse de la température. Dans les cas graves, le choc s'installe rapidement vers le 4e-5e jour, tout de suite après ou en même temps que les manifestations hémorragiques. L'issue est fatale dans 15% à plus de 50% des cas. Sinon la convalescence est généralement rapide et sans complications. Le foie est palpable dans la moitié des cas hospitalisés en Thaïlande et en Malaisie, mais ce symptôme ne s'observe pas aux Philippines; l'épistaxis qui n'est ni fréquente ni grave en Thaïlande, en Malaisie et au Viet-Nam l'est en revanche aux Philippines. La gravité de la maladie et la mortalité sont très variables suivant les épidémies.

Aucune méthode clinique ou de laboratoire ne permet actuellement de distinguer rapidement les cas bénins de fièvre hémorragique de la dengue classique. Le diagnostic virologique éventuel permet ultérieurement de définir la maladie. Les observations nécropsiques se limitent habituellement à constater des hémorragies au niveau de divers organes, une réaction réticulo-endothéliale, un arrêt de la maturation des mégacaryocytes ou une hypoplasie cellulaire de la moelle osseuse, des lésions dégénératives du foie ou une infiltration de l'organe, une pneumonie interstitielle. Les résultats des examens anatomopathologiques sont d'ailleurs très variables suivant les régions.

Dans certains cas confirmés de fièvre hémorragique grave, on note, après 3 jours ou plus de fièvre, l'apparition d'un collapsus cardio-vasculaire et/ou d'une hémorragie gastro-intestinale. Des études faites en Thaïlande attri-

buent ces symptômes à une perméabilité anormale des capillaires entraînant une hémoconcentration avec acidose légère. Le travail du cœur est augmenté, une hypoxie tissulaire s'installe, augmentant l'acidose et aggravant les lésions capillaires. De graves hémorragies gastro-intestinales peuvent également accompagner le choc. Certains malades présentent une forte thrombocytopénie par arrêt de la maturation des mégacaryocytes, ou destruction accrue des plaquettes sanguines. Dans les formes graves, on note une leucocytose, alors que dans la dengue classique, on observe habituellement une leucopénie tardive. Par ailleurs, les troubles de l'hémostase n'expliquent pas entièrement les manifestations hémorragiques. Parmi d'autres signes, il faut citer l'hépatomégalie, fréquente en Thaïlande, des pneumonies interstitielles et des épanchements pleuraux visibles aux rayons X, et chez les enfants, une irritabilité marquée avec anomalies transitoires de l'électro-encéphalogramme.

L'extension de la fièvre hémorragique à des zones auparavant indemnes semblerait prouver que la maladie est due à un ou des virus mutants. A Calcutta, on l'a attribuée à un virus de la dengue du type 2, tandis qu'à Vellore, le type 2 ne provoque que la dengue classique. A Bangkok, on a relevé des différences antigéniques entre des virus du même type et l'on a supposé que les souches responsables des fièvres hémorragiques graves seraient plus proches des virus TH-36 (dengue du type 5) et TH-Sman (dengue du type 6) que des virus de la dengue des types 1 et 2. Une autre hypothèse fait de la fièvre hémorragique une sorte de réaction d'hypersensibilité. Les sérums des malades atteints de fièvre hémorragique grave sont anormalement riches en anticorps et chez eux, le virus de la dengue disparaît plus rapidement du sang et des tissus que chez ceux qui présentent une forme bénigne; du point de vue épidémiologique, la fièvre hémorragique est souvent attribuée à des virus de la dengue de types très divers et beaucoup de patients possèdent accessoirement des anticorps vis-à-vis de nombreux virus du groupe B. Dans d'autres parties du monde, les épidémies de dengue ne s'accompagnent ni d'hémorragies ni de choc, et dans les zones où la fièvre hémorragique est endémique, les étrangers ne présentent que les formes classiques de la dengue lorsqu'ils sont infectés par les virus locaux. On a enfin invoqué le rôle de facteurs héréditaires ou de l'état nutritionnel.

Plusieurs méthodes de traitement du choc ont été proposées. Leur comparaison est malaisée en raison du petit nombre de sujets traités et de la gravité de la maladie qui rend difficiles des essais contrôlés.

Le diagnostic étiologique ne peut que rarement être établi par les méthodes sérologiques, la majorité des malades ayant subi antérieurement des infections par des virus proches. Ces méthodes sont décrites dans le mémoire et font l'objet d'une revue critique de leurs avantages et inconvénients respectifs. Les virus de la dengue ont toujours été difficiles à isoler et les souches produisant les syndromes hémorragiques le sont particulièrement. Deux hypothèses sont avancées pour expliquer cette particularité: les souches très pathogènes pour l'homme seraient peu virulentes pour la souris; ou bien, les anticorps apparaîtraient plus rapidement chez les malades graves et pourraient neutraliser le virus même au cours de la phase aiguë de l'affection. Le succès des isolements dépend de la précocité des prélèvements de sérum; ils sont rarement positifs à partir de sang ou de tissus prélevés à l'autopsie. Le mémoire expose les méthodes classiques ou plus récentes d'isolement des virus par inoculation aux souriceaux à la mamelle ou d'identification sur cultures de tissu.

Aedes aegypti est le principal vecteur des virus de la dengue et du virus chikungunya dans les régions où la fièvre hémorragique est observée. Ces virus ont été isolés d'*A. aegypti* naturellement infectés; la distribution dans l'espace et dans le temps du moustique coïncide avec celle de la maladie; la transmission expérimentale de l'affection a fait l'objet de recherches limitées. Le rôle primordial d'*A. aegypti* n'exclut pas celui d'*A. albopictus* qui est largement répandu. On doit aussi envisager la possibilité que les virus de la dengue soient responsables de certaines zoonoses. L'existence d'un cycle animal du virus chikungunya, auquel participeraient des *Culex*, est très vraisemblable. La fièvre hémorragique épidémique est une maladie de la saison des pluies, mais elle peut durer toute l'année dans les régions d'hyperendémicité. La transmission d'une région à une autre s'effectue probablement par l'intermédiaire de personnes en période d'incubation et le transport d'*A. aegypti* ou de personnes infectées par les avions peut permettre la propagation des virus d'un pays à un autre.

Dans les zones urbaines, les mesures préventives se limitent à la lutte contre le vecteur, *A. aegypti*, mais le gène porteur de la résistance au DDT est maintenant largement répandu dans les populations d'*A. aegypti* de l'Asie du Sud-Est et du Pacifique occidental. Aussi la coopération du public est-elle d'une extrême importance ainsi que l'éducation sanitaire. Les programmes d'envergure nécessitent une préparation soigneuse et de nouvelles enquêtes. Des possibilités de lutte sont offertes par l'emploi des moustiques mâles stérilisés, les méthodes génétiques ou l'utilisation de parasites ou de prédateurs.

Malgré les études de ces dernières années, l'importance réelle du problème de la fièvre hémorragique n'est pas encore connue. Des nouvelles enquêtes sérologiques sont nécessaires. Les sérums devraient être adressés aux laboratoires OMS de référence et d'autres laboratoires spécialisés; un organisme international centraliserait et diffuserait les renseignements sérologiques et les observations épidémiologiques. Dans chaque pays, les ports et le périmètre des aéroports devraient être débarrassés d'*A. aegypti*, conformément au Règlement sanitaire international. Il n'existe malheureusement aucun vaccin prophylactique à l'heure actuelle.

De nouvelles recherches sont nécessaires dans de nombreux domaines: méthodes d'isolement et de typage des virus de la dengue; différenciation des virus responsables de la dengue classique et des virus provoquant un

syndrome hémorragique grave; étude des facteurs propres à l'hôte, de la pathogénie de l'affection, et de la pathophysiologie du choc. Il faut aussi approfondir l'étude de la dengue classique dans les régions où la fièvre hémorragique n'existe pas; le rôle d'*A. albopictus* dans la transmission doit être comparé à celui d'*A. aegypti*, et *Culex tritaeniorhynchus* et *Culex gelidus* étudiés en tant que vecteurs des virus chikungunya; un réservoir animal sylvatique des virus de la dengue doit être recherché. Pour réaliser une prévention efficace, les recherches doivent encore porter sur l'obtention de vaccins, l'écologie d'*Aedes aegypti*, les épreuves de laboratoire et l'ensemble des critères de diagnostic de la dengue et des fièvres hémorragiques.