diagnosis of haemorrhgic fever and from 265 well children, surgical patients or patients with miscellaneous febrile illnesses seen at the Out-patient Department, Children's Hospital and maternal and child health centres in Bangkok. The age, ethnic and sex composition of the two compared groups were nearly identical. Specimens were tested for blood groups A, B, O, AB, M, N, MN, and Rh phenotypes C, D, E, c, e, qualitative glucose-6-phosphate dehydrogenase deficiency and haemoglobin type. Plasmas will be tested for haptoglobin and transferrin type and Gm and Gc proteins. Analysis of the plasmas and Rh system is not complete at the time of writing. No difference was noted in the frequency of G-6-PD deficiency or haemoglobin type between the two groups. There were slight maldistributions in A, B, MN and blood group antigens in haemorrhagic fever patients (A = 19.2%, B = 38.5%, MN = 60.8%, M = 29.0%) as compared with controls (A = 26.2%, B = 31.7%, MN = 66.9%, M = 18.5%). These differences were not significant by the χ^2 test. Studies are continuing.

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Attempts at Virus Recovery from Patients Dying of Thai Haemorrhagic Fever *

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The fatality rate among hospitalized cases in outbreaks of Thai haemorrhagic fever usually varies between 5% and 10%. Patients who survive severe disease almost invariably present serological evidence of recent dengue infection. On the other hand, patients who have clinical haemorrhagic fever and do not survive do not always show serological evidence of dengue infection. This study reports on attempts to isolate dengue virus from blood specimens and organs obtained from 102 fatal cases of haemorrhagic fever occurring in 1962-64. The age and sex distributions of these cases were representative of the age and sex distribution of hospitalized cases and deaths due to haemorrhagic fever in Bangkok and Thonburi for the years studied.

In 1962-63, samples of venous blood obtained before death or of heart blood obtained immediately after death were collected from 46 children who died of haemorrhagic fever between the 1st and the 7th day after onset of the fever; 37 of these specimens were obtained 3-5 days after onset of the illness. Samples of sera or plasma were inoculated into suckling mice as described by Singharaj, Simasathien & Halstead.[1] Dengue viruses were recovered from two specimens, collected on the 1st and on the 3rd day of illness, respectively. The virus isolated from the former was tentatively identified as dengue type 1 and that from the latter as dengue type 2. In 1964, 12 specimens of heart blood were tested at 1 : 4, 1 : 10, 1 : 100 and 1 : 1000 dilutions in mice and in BS-C-1 cells. A dengue type 2 virus was recovered from one specimen at a serum dilution of 1 : 10.

Autopsy specimens were obtained from patients expiring in Bangkok or Thonburi hospitals. In most instances the bodies were moved to a refrigerator immediately after death. In 46 autopsy specimens studied in 1962-63, the interval between death and autopsy varied between 6 and 72 hours; in 38 of these cases the autopsies were performed within 24 hours of death. Tissues from patients were transferred to the laboratory on wet ice, were quick-frozen and were then stored at −70°C. Each organ was weighed, ground with Alundum, diluted to a 10% suspension with phosphate buffered saline containing bovine albumin (0.75%) and antibiotics, centrifuged and inoculated into mice. Suspensions of materials from 10 autopsies carried out in 1964 were diluted 1 : 5, 1 : 10, 1 : 100 and 1 : 1000 and inoculated simultaneously into suckling mice and BS-C-1 cells. The organs concerned were mainly the major viscera. No dengue viruses were isolated from 248 specimens tested in mice or tissue culture between 1962 and 1964. Agents that were pathogenic for suckling mice were recovered from the heart tissue of a 3-year-old Thai boy who died on the 3rd day of illness and from the lung tissue of a 5-year-old Chinese girl who died on the 5th day of

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[1] See page 66.

disease. These viruses were ether-resistant and produced lesions in suckling mice consistent with coxsackie A viruses. No dengue viruses were isolated from 16 liver specimens obtained immediately after death by means of a Vim-Silverman needle. Fresh liver tissue was trypsinized and inoculated into BS-C-1 cells as well as into tubes containing growth medium only.

The results described differ from those obtained by Dasaneyavaja & Charanasri,[2] who reported the isolation of a dengue type 4 virus from the liver tissue of a 4-year-old Chinese girl who died of haemorrhagic fever in 1960. They are consistent, however, with the observations of Bhamarapravati & Boonyapaknavik,[3] who were unable to demonstrate dengue antigen in human tissues stained with fluorescein-conjugated antidengue globulin and with our own failure to protect mice against dengue virus challenge by immunizing them with suspensions of autopsy materials. The absence of demonstrable etiological agent or antigen in the tissues of patients dying of an infectious disease of relatively short course is puzzling. Such a phenomenon might be explained by one or more of the following hypotheses: (*a*) dengue virus may proliferate in tissues other than those conventionally examined; (*b*) the virus may be thermally inactivated before inoculation into the laboratory host; (*c*) toxic substances (excluding neutralizing antibody) in autopsy materials may inactivate dengue virus; (*d*) fatal haemorrhagic fever may be caused by viruses which do not readily propagate in mice or tissue culture; (*e*) some illnesses diagnosed as haemorrhagic fever and terminating fatally may be caused by agents other than dengue viruses; (*f*) some deaths from haemorrhagic fever may be itrogenic; and (*g*) dengue viruses may be irreversibly inactivated by antibody before or during the severe stages of haemorrhagic fever and before death. Further virological, clinical and pathological studies will have to be carried out before these and other questions can be answered.

[2] Dasaneyavaja, A. & Charansri, U. (1962) In: *Symposium on Haemorrhagic Fever 1961*, Bangkok, p. 61 (SEATO Medical Research Monograph No. 2).

[3] See page 50.

Recent Virological Studies of Philippine Haemorrhagic Fever*

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This paper presents the results of some virological studies on blood samples collected in the Philippines between November 1963 and August 1964.

Most of the blood samples were taken during the febrile stage from hospitalized patients diagnosed as suffering from haemorrhagic fever or influenza. Samples were likewise taken from apparently normal adults and children in several places in the country.

BS-C-1 cells were used for most of the virus isolation and neutralization techniques. Some samples were also inoculated into suckling mice for virus isolation. The standard haemagglutination-inhibition (HI) and complement-fixation (CF) techniques adapted to microvolumes were used throughout these studies.

The results may be summarized as follows:

1. Fifteen paired sera from hospitalized cases of clinically diagnosed haemorrhagic fever from Manila and surrounding provinces were tested by HI and CF tests. Fourteen showed HI and CF antibodies for dengue virus. One had no demonstrable antibody for either dengue or chikungunya virus and none of the remaining fourteen showed antibody for chikungunya.

2. HI tests on single serum samples from 55 clinically diagnosed influenza cases showed that 65% had antibody for dengue virus, 12.74% having titres that varied from 1 : 640 to 1 : 5120. 6.94% had HI antibody for chikungunya virus.

3. HI tests on single serum samples from 31 clinically diagnosed haemorrhagic fever cases showed that 80.64% had antibodies for dengue virus, 35.5% having a titre of at least 1 : 1280. No antibody for chikungunya virus was detected.

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