of 1 day, or of 3 to 4 days in the case of the last two doses. Interruption of pregnancy was complete (2/2 and 9/9) in animals treated with doses of 4 and 2 mg, respectively, but was incomplete (1/4, 3/7, and 2/8, respectively) in the remainder. Intravenous injection of 0.25 mg of 5-HT in three mice at the same stage of pregnancy had no effect.

Thus it is obvious that 5-HT, even in small doses, can interrupt pregnancy. In such cases the uterus appeared dark, and in some cases almost black, in color at laparotomy. When the uterus was opened, the placentas showed gross hemorrhagic changes, mostly involving the fetal part, while the amniotic sac contained the reabsorbing fetus in the form of a yellow gelatinous material. In some of the animals that had received the smaller doses of 5-HT and in which pregnancy appeared normal, minute hemorrhages in the placentas were nevertheless obvious to the naked eye. Histological examination of the placentas of animals in which pregnancy had been disturbed revealed massive collections of red blood corpuscles just under the surface and disorganization of the normal villous pattern (see Fig. 1).

In an attempt to determine how rapidly 5-HT could produce deleterious changes in the uterine contents, nine mice in an advanced stage of pregnancy (17 days) were injected subcutaneously with a single dose of 1 or 2 mg of 5-HT and examined at intervals ranging from ½ to 3½ hours after the injection. In two animals injected, respectively, with 2 and 1 mg of 5-HT and examined ½ hour later, there were obvious hemorrhagic changes in all the placentas, and all the fetuses were already dead. Some of the fetuses in animals examined later were still alive, but the placentas of these animals showed hemorrhagic changes, and the placentas of all animals killed 2 or more hours after the administration of 5-HT showed marked hemorrhagic changes. It is obvious, therefore, that 5-HT can very rapidly produce hemorrhage and disorganization of the placenta, leading to death of the fetus.

Table 1. Effect of 5-HT and iproniazid at various stages of pregnancy in mice. The ratios represent the number of animals found pregnant out of the total number of animals used.

Period of treatment (day No.)	5-HT (1 mg/day)		Iproniazid (5 to 10 mg/day)	
	Control	Treated	Control	Treated
1–6	19/27	4/27	13/26	5/24
3–8	7/12	1/12	12/14	2/14
6–11	15/26	8/26	12/20	0/15
11–16		0/11*	9/13	9/13†

* All animals were pregnant on the 11th day —that is, before treatment was started.
† Three litters were born dead at full term.

Experiments on rabbits gave rather similar results, including hemorrhage in the placenta. The most marked effects were seen late in pregnancy.

The fact that relatively small doses of 5-HT can interrupt pregnancy and produce in the placenta hemorrhages which simulate findings in the human placenta in the toxemia of pregnancy [where red infarcts are constantly observed (*5*)] raises the question whether 5-HT is involved in the pathogenesis of toxemia (*6*).

EVELYN POULSON
M. BOTROS*
J. M. ROBSON
Department of Pharmacology, Guy's Hospital Medical School, London, England

References and Notes

1. A. Pletscher, P. A. Shore, B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **116**, abstr. 46 (1956).
2. R. Gaunt, A. A. Renzi, N. Antonchak, G. J. Miller, M. Gilman, *Ann. N.Y. Acad. Sci.* **59**, 22 (1954); H. Tuchmann-Duplessis and L. Mercier Parot, *Compt. rend.* **243**, 410 (1956).
3. H. Weissbach, thesis, George Washington University (1957).
4. J. M. Robson, *Mem. Soc. Endocrinol.* **6**, 54 (1959).
5. N. M. Falkiner, *Irish J. Med. Sci.* **347**, 81 (March 1942).
6. This study was assisted by a grant to the senior author from the British Medical Research Council.
* Present address: Ein-Shams University, Cairo, Egypt.

14 December 1959

Viruses Associated with Epidemic Hemorrhagic Fevers of the Philippines and Thailand

Abstract. Epidemiologic, clinical, and etiologic studies were carried out on a newly recognized, frequently fatal, pediatric disease syndrome which occurred in urban areas infested with *Aedes aegypti* mosquitoes. Four types of dengue virus (two of which are new), chikungunya virus, and another virus yet to be identified were isolated from the blood of patients. Dengue viruses, types 2 and 3, were isolated from the mosquitoes. Ample serologic confirmation was obtained of concurrent hemorrhagic fever and infection with one or more of these viruses. Thus, it was discovered that viruses of previously recognized types and of closely related new types apparently have etiologic roles in a new and highly dangerous epidemic disease syndrome.

Pediatricians observed a number of cases of a serious, frequently fatal, hemorrhagic, febrile disease in Manila, Luzon, in the rainy season of 1954. It was described as a new disease entity and named Philippine hemorrhagic fever (*1*). During the rainy season of 1956, a large epidemic occurred in Manila. This coincided with a search for the arthropod-borne [ar-bo (*2*)] viruses of the area that was being made by a field research unit (*3*) under the direction of one of us (W.M.H.) Observations of a clinical, epidemiologic and laboratory nature on hemorrhagic ("H") fever were included in the study, though no relation to arthropod transmission was then recognized.

Over 750 cases of "H" fever were reported in Manila during the rainy-season months of July through October; approximately 10 percent of the cases were fatal. With few exceptions the disease occurred in children, predominantly below 6 years of age; all were orientals, in urban or suburban areas where *Aedes aegypti* were present in relatively large numbers. Cases were not found where *A. albopictus* but not *A. aegypti* were present. The disease, although having some similarities to the epidemic hemorrhagic fever of Korea and Manchuria, could be readily differentiated clinically; also, it did not closely resemble yellow fever, leptospirosis, or Omsk or Crimean hemorrhagic fevers.

As a result of a preliminary report (*4*) on the disease, one of us was requested to study an alarming epidemic which occurred in and near Bangkok during the rainy season of 1958. Here again a distinctive hemorrhagic disease had been first recognized in 1954, occurring as a small epidemic among children, and a few cases had been observed annually thereafter at the same season. Approximately 2500 hospitalized cases and 250 deaths marked the major epidemic observed and reported here. Clinically the disease resembled rather closely that observed in Manila, but it differed in significant ways; this led the clinical observer of cases in both areas (W.M.H.) to expect to find a related but different etiologic agent. The age and race of the patients and the seasonal and urban distribution of the diseases in Thailand and the Philippines, were similar, as well as the marked association with *A. aegypti.*

Mosquitoes from the area and blood sera from patients in the acute and convalescent phases of the disease were collected, frozen in dry ice, and transported to Pittsburgh. Sera, in several dilutions, from patients in the acute phase and mosquito suspensions by species were inoculated intracerebrally into suckling mice and passaged blindly when necessary. Because of the possibility suggested by epidemiologic findings that dengue-related viruses might be involved, the surviving mice were challenged with dengue virus type 2,

Table 1. Antigenic pattern of dengue viruses, including new types, by neutralization and by complement fixation tests.

Dengue virus type	Neutralization index, monkey sera, by type				Complement fixation titer, mouse sera, by type			
	1	2	3	4	1	2	3	4
1	5400	170	160	140	16	8	4	<4
2	10	1000	72	27	<4	128	<4	<4
3	4	2	1000	3	4	32	32	4
4	4	2	1	>4350	<4	8	8	32

and immunity was frequently demonstrated.

From the Manila area, 14 viruses were adapted to suckling mice; in each case adaptation required 10 to 20 passages. Incubation periods shortened from 20 or more days to 3 or 6 days, and titers rose from $<10^0$ to 10^4 or 10^6 LD_{50}. These all proved to be antigenically related to dengue viruses types 1 and 2 (*5*) but, except for a single type 2 strain, represent new types. Designation as dengue types 3 and 4 is recommended. Antigenic relations of the group as determined by complement fixation and by neutralization are shown in Table 1 (*6*). Human sera yielded nine type 3 and two type 4 viruses, and one type 2 virus, while *Aedes aegypti* and *Culex tritaeniorhynchus* each yielded one type 3 virus; these were the first dengue viruses to be reported from wild-caught mosquitoes.

From Bangkok, agents were isolated with less difficulty both from human sera and from *Aedes aegypti.* Of nine isolations made and identified in our laboratory, six were found, by neutralization and complement fixation tests, to be similar antigenically to dengue type 2, and three, to chikungunya, a group-A virus causing a dengue-like febrile disease in Africa and apparently transmitted there by *A. aegypti* (*7*). Three isolations typed as dengue 2 were from *A. aegypti,* while all other dengue and chikungunya viruses were from serum. Among viruses isolated from serum by other workers in Bangkok and sent to us for identification were two of the chikungunya type (*8*) and one tentatively identified as dengue type 1 (*9*). One of our isolations from human serum behaves like still another agent. Thus, three or possibly four virus types have been isolated. Other sera are still under test.

Serological studies of patients are as yet incomplete, but by demonstrating a rise of antibody titer in suitably spaced paired specimens in a combination of neutralization, complement fixation, and hemagglutination-inhibition tests, we have obtained ample confirmation of concomitant infection with one or more of these viruses and the disease syndrome. A number of patients from Bangkok showed a simultaneous rise in titer in response to both chikungunya and dengue viruses. A few patients having brief, principally mild, illnesses, without shock, showed no rise in antibody titer in response to the group A or B agents employed.

It is too early to evaluate completely the possible etiological significance of all these agents isolated from blood sera of patients with the broad clinical syndrome of "H" fever and from mosquitoes of the two epidemic urban areas. However, it is difficult to draw any conclusion other than that of probable etiologic relationship when the following points are considered: (i) viruses of the same type were isolated from several patients with the same syndrome; (ii) homologous rises in antibody titer were demonstrated in these and in numerous other patients; (iii) the epidemic pattern of the disease, with seasonal occurrence and *A. aegypti* association, was similar to that expected for related agents; (iv) several of the agents were isolated from *A. aegypti* mosquitoes; (v) no classical dengue-like disease was observed simultaneously in the areas; and (vi) older persons in these communities were probably immune to these or closely related agents; this would explain the unusual age distribution for the observed disease. Such immunity had been partially demonstrated in previous surveys, dengue antibody having been found in Manila (*10*) and Semliki Forest virus (related antigenically to chikungunya) antibody in Bangkok (*11*).

The next question of major interest which these observations raise is the adequacy of antigenic analysis, now generally accepted as the final method of identification for the ar-bo virus group. Several of the incriminated agents are identical with, or very closely related antigenically, to agents causing either classical dengue (dengue types 1 and 2) or chikungunya disease (bent bones). The "H" fevers do not resemble the classical dengue syndrome. It is true, however, that in association with a few epidemics called dengue on a clinical basis, an occasional hemorrhagic case, sometimes fatal, has been reported. Such cases could either have represented other infections or have been an indication that hemorrhagic variants of the common viruses occasionally arise. Sabin (*12*) noted occasional petechiae in cases of dengue among volunteers. Further study is obviously required.

W. McD. Hammon
A. Rudnick
G. E. Sather

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

References and Notes

1. F. N. Quintos *et al., Philippine J. Pediat.* **3**, 1 (1954).
2. We now prefer this abbreviation for the term *arthropod-borne zoonoses viruses* to the commonly used "arbor," thought erroneously by many to be of Latin derivation and to mean tree.
3. This investigation was carried out in cooperation with the American Armed Forces; the Philippine Health Department; the School of Hygiene, University of the Philippines; and the Philippine National Museum. The work was done in part under the sponsorship of the Commission on Viral Infections, Armed Forces Epidemiological Board, and was supported by the Office of the Surgeon General, Department of the Air Force, by the Office of the Surgeon General, Department of the Army, and by a research grant from the National Institutes of Health, U.S. Public Health Service.
4. W. McD. Hammon *et al., Abstr. 9th Pacific Sci. Congr. Bangkok* (1957), p. 187.
5. ———, *Abstr. 6th Intern. Congr. Trop. Med. and Malaria,* Lisbon (1958), p. 163.
6. These relationships were subsequently confirmed by hemagglutination inhibition and by complement fixation by Jordi Casals of the Rockefeller Foundation.
7. M. C. Robinson, *Trans. Roy. Soc. Trop. Med. Hyg.* **49**, 28 (1955); W. H. R. Lumsden, *ibid.* **49**, 33 (1955).
8. The viruses were isolated by Sriprapai Phong-Aksara of the Queen Saovabhas Memorial, Pasteur Institute, Bangkok, Thailand.
9. The virus was isolated by Sman Vardhanabhuti, Faculty of Medicine in Chiengmai, Siriraj Hospital, Dhonburi, Thailand.
10. W. McD. Hammon *et al., Am. J. Trop. Med. Hyg.* **7**, 323 (1958).
11. W. L. Pond *et al.,* paper presented at the 9th Pacific Science Congress, Bangkok (1957).
12. A. B. Sabin, *Am. J. Trop. Med. Hyg.* **1**, 30 (1952).

19 November 1959

Species Differentiation of Insects by Hemolymph Electrophoresis

Abstract. By means of microelectrophoresis in agar gel, we studied the hemolymph proteins of different species of the family Triatomidae (class Insecta) and different species of the families Ixodidae and Argasidae (class Arachnoidea). The results show a specific electrophoretic pattern for each species. Insects infected with pathogenic organisms have the same pattern as normal insects.

The hemolymph of blood-sucking insects has a high protein content (7 to 8 gm-percent by refractometric determination); 2 to 3 μl hemolymph are easily taken by cutting a leg of the in-