Etiology of the 1927-28 Epidemic of Dengue in Greece. (25474)

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In 1927 and 1928, an extensive epidemic disease diagnosed as dengue occurred in Greece. It has been estimated that the total number of cases probably exceeded one million(1). The vector was, in all probability, *Aedes aegypti*, which was very prevalent. The diagnosis of dengue was made entirely on clinical grounds, since no satisfactory susceptible experimental animal was available at that time. During recent years, several viral agents have been established in mice from cases diagnosed as dengue. Two of these are known as dengue types I and II(2,3). These viruses are distinct from each other, although there is quite a marked immunological overlap. Investigators in Israel have shown that infections in man with the West Nile virus may produce a disease which clinically resembles dengue(4,5,6). Infections with West Nile virus are exceedingly common in the eastern Mediterranean, not only in Israel but more particularly in Egypt(7). In the latter country the infection is endemic, producing as a rule only a mild febrile disease in young children. Limited serological investigations in these laboratories have not shown infections with either type I or type II dengue in Egypt. The reason for this is probably the scarcity of *Aedes aegypti* in that country. No information concerning the presence of dengue in Israel is known to the authors. The 2 types of dengue and the West Nile virus belong to Casals' group B of immunologically related arthropod-borne viruses. An agent which has recently been isolated in Tanganyika and shown to be the cause of an epidemic, dengue-like disease, is a member of Casals' group A (8,9). These 2 groups of agents are completely distinct immunologically. It was thought of interest to undertake serological investigations with sera obtained from residents of Athens to determine, if possible, the etiology of the "dengue" epidemic which occurred in 1927-28. In these studies, only the possible role of group B agents as the cause of the dengue epidemic was considered.

Materials and methods. In 1958, 56 serum specimens were obtained from Athenian residents ranging in age from 2½ to 70 years. These can be divided into 2 groups. The first consisted of 27 specimens obtained from individuals who were 32 or more years old and consequently were living at the time of the epidemic. The second group consisted of 29 specimens obtained from individuals who, at the time of bleeding, were 29 years of age or younger and thus had been born subsequent to the epidemic. For determining the presence or absence of antibodies, main emphasis was placed on hemagglutination-inhibition (HI) tests(10). All sera were tested against 7 different group B virus antigens: West Nile (strain IG 2266), dengue types I and II, Japanese encephalitis, yellow fever (Asibi), Russian spring-summer encephalitis (RSSE) and SA H 336. The last named virus is an agent closely related to Uganda S(11).

Results. HI tests. The HI antibodies produced following a group B arthropod-borne virus infection are not specific in the sense that only homologous antibodies are developed. Antibodies to related viruses are almost invariably produced, particularly with the passage of time; these antibodies are, as a rule, at lower titers.

In the case of the present sera, the results of the HI tests, as judged by the number of sera positive against each antigen as well as by the relative titers that each serum gave against the different antigens, led to the conclusion that infection with dengue type I was in all probability responsible for the positive reactions. Previous experience with dengue type I infections in rhesus monkeys had indicated clearly that this infection leads to production of HI antibodies capable of reacting in high titer with a wide variety of group B antigens. In the present study, the highest

TABLE I. Results of Hemagglutination-Inhibition Tests.

Age	No. of sera	No. negative	No. positive	% positive
0- 9	14	13	1	
10-19	11	11	0	3
20-29	4	4	0	
30-39	4	2	2	
40-49	5	2	3	67
50+	18	6	12	

titers of HI antibodies were obtained, as a rule, with a dengue type I antigen. In several instances, however, titers obtained with the IG 2266 strain of West Nile virus were higher than those obtained with the dengue type I antigen. Although these results seemed to suggest that some of the infections in Athens might have been due to West Nile virus, it is known from extensive studies on Egyptian sera that the HI pattern following a West Nile infection is characterized by high titers with West Nile and low titers with the 2 strains of dengue, a pattern quite distinct from that observed in the present study.

The results with dengue type I and West Nile antigens indicated that only one of the 29 sera in the younger age group was positive, whereas 17 of 27 (67%) in the older age group were positive. The results are summarized in Table I.

All sera that reacted did so against dengue type I and West Nile antigens, the mean titer with both antigens being 1:80. With 8 sera, the titer was higher against dengue type I; in 5 cases, higher against West Nile, and in 5, the titers were the same.

The HI titers obtained with the type I dengue ranged from 1:10 to 1:1280, whereas the range with the West Nile antigen was from 1:10 to 1:640. Eight units of antigen were used in all cases.

With one exception, all the positive sera gave the highest inhibition with a dengue type I or West Nile antigen. The exception was the serum obtained from an individual born in 1899. This serum reacted in a dilution of 1:80 with a RSSE antigen and in a dilution of only 1:10 with dengue type I and West Nile antigens. This finding suggested that the individual had been infected with RSSE virus.

Neutralization tests. To obtain further information, neutralization tests with dengue type I and West Nile viruses were done. As some of the specimens had been used up, the number of sera which could be tested in this manner was limited. Mixtures of sera and virus were incubated for 1 hour at 37°C before intracerebral inoculation into young adult mice. Titrations of the virus preparations used in the tests indicated that the virus dose per mouse was 40 LD_{50} for the West Nile virus and 50 LD_{50} for the dengue type I. The results are shown in Table II.

All 28 sera in the test were negative in the neutralization test with the West Nile virus, whereas 10 gave clear-cut protection with the dengue type I virus. The one serum in the younger age group that gave a positive reaction in a 1:10 dilution in the HI test with a West Nile but not with any other antigen, failed to exhibit any neutralizing action against either the West Nile or the dengue type I virus. It is possible that the positive reaction obtained with this serum in the HI test is due to a technical error. All 10 sera positive in the dengue neutralization test were also positive in the HI test. One of these had shown a higher HI titer with a West Nile antigen than with a dengue type I antigen. The correspondence between the results

TABLE II. Results of Neutralization Tests.

Results of HI tests	Age group	No. of sera	Dengue type I		West Nile	
			No. negative	No. positive	No. negative	No. positive
Negative	0-29	8	8	0	8	0
	32-70	9	9	0	9	0
Positive	0-29	1	1	0	1	0
	32-70	10	0	10	10	0
Total		28	18	10	28	0

obtained with the HI and the neutralization tests with dengue type I virus was remarkably good.

As mentioned above, the HI studies had indicated the possibility that one of the donors had undergone an infection with RSSE virus. In a neutralization test with RSSE virus, this serum neutralized 300 LD_{50} of the virus, whereas 3 HI negative sera showed no neutralizing action. The donor of this serum had lived all his life in Greece.

These results indicate clearly that the epidemic of dengue in Athens in 1927-28 was, in all probability, due to a dengue type I virus. There is no evidence that any West Nile infections occurred. The high titers obtained in HI tests with a West Nile antigen are possibly the result of the high reactivity of the antigen used. Antigens prepared with the IG 2266 strain of West Nile virus used in these studies have consistently reacted to a higher titer with antisera than antigens prepared from other strains of West Nile.

Discussion. These studies have provided a unique opportunity for acquiring information about the persistence of HI antibodies following a group B arthropod-borne virus infection. In most regions where these agents occur, the infections are endemic, and individuals are exposed to repeated infections by one or more group B agents. In the present instance, however, the complete absence of any group B antibodies in the sera of the younger age group shows that no group B arthropod-borne virus infection has occurred in Athens since the epidemic of 1927-28. Under these circumstances, the close correspondence between the results obtained in the HI and neutralization tests is good evidence that HI antibodies persist for at least 30 years after a dengue type I infection.

Summary. The results of serological investigations on sera obtained from residents of Athens, Greece, indicate clearly that the epidemic of dengue which occurred in that country in 1927-28 was, in all probability, due to type I dengue virus. Antibodies were demonstrated in approximately 70% of sera obtained from persons living at the time of the epidemic. Sera from individuals born subsequent to the epidemic were negative.

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Absorption and Urinary Excretion of Chloramphenicol and 2 Analogues, Thiocymetin and U-15,442 in Normal Men.* (25475)

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Following the discovery of chloramphenicol and elucidation of its chemical structure, numerous derivatives were prepared in different laboratories. However, in the various test systems employed, none of them have shown

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