tatively, of the country and (with a view to exports) of the world. For example, what is needed to keep all the people as physically fit as their natures will permit? (2) A great development of adult education, fitting the people to do the needed work. The astonishing success of the war workers shows how adaptable people are, if really interested and efficiently taught.

T. D. A. COCKERELL

SPECIAL ARTICLES

PRODUCTION OF IMMUNITY TO DENGUE WITH VIRUS MODIFIED BY PROPAGATION IN MICE[1]

THE purpose of this preliminary communication is to report, (a) the successful propagation of dengue virus in mice, and, (b) that beginning with the 7th passage in mice the virus had undergone such extensive modification in its pathogenic properties for human beings that consideration of its use as a vaccine seemed justifiable.

The work to be reported here is part of a systematic investigation of the virus of dengue with special emphasis on those of its properties which might lend themselves to the development of a vaccine. The work was carried on with five strains of dengue virus isolated by one of us (A.B.S.) from outbreaks of the disease in Hawaii and New Guinea.[2] The identity of the virus was established (a) by its capacity to produce in human volunteers a disease characterized by a fever of 4 to 7 days' duration, severe pain in various parts of the body, rash, lymphadenopathy and leukopenia with certain changes in the leukocytic formula, and (b) by its transmission after a suitable extrinsic incubation period by relatively small numbers of *Aëdes aegypti* mosquitoes.[3] Most of the studies were carried out with the Hawaiian strain of the virus for the following reasons: (a) it was the most virulent in the sense that it produced the most severe disease in human volunteers; (b) about 1,000,000 minimal human infective doses per cc were found in the serum of experimental cases during the first 24 hours of the disease,[4] and (c) having found that it had a particle size in the range of 20 mμ it was possible to obtain concentrates of the virus possessing more than 10,000,000 minimal human infective doses per cc by centrifugation at 24,000 r.p.m. for 90 minutes in an 8-inch rotor.[4,5] With these concentrated preparations of virus an exhaustive effort was made to obtain growth in cultures containing mouse embryo tissues and in embryonated chicken eggs of various ages, inoculated by various routes and incubated for varying periods at different temperatures, but with negative results. After 2 or more passages, the cultures produced neither disease nor immunity upon inoculation in human beings.

Dinger and Snijders[6] in 1931 attempted to propagate dengue virus from the Dutch East Indies by intracerebral inoculation of mice, but without success. Our own initial attempts to infect young mice in this manner with human serum of proved infectivity were also regarded as negative, but it must be stated that the mice were observed only for gross signs of involvement of the nervous system for the usual period of 2 to 3 weeks. In the first two attempts with concentrated, ultracentrifuged Hawaii virus, there was an occasional mouse that died or exhibited signs of nervous system involvement, but after one or two further passages negative results were obtained. On the third attempt with intracerebral inoculation of concentrated, ultracentrifuged Hawaii virus in 10 to 12-day old Swiss albino mice, it proved possible to transmit in series an agent which produced vaguely discernible to severe signs of nervous system involvement in a varying, and initially very small, proportion of the inoculated mice. However, 16 serial passages have now been completed and the tests with mouse-passaged material in human volunteers have established that the virus propagated in mice is dengue. Only 10 to 20 per cent. of the inoculated mice at first exhibited clinical signs of the infection (sometimes limited to slight weakness of the extremities demonstrable only by special tests), and the incubation period was frequently 3 to 4 weeks. Beginning with the 6th passage the incubation period

[1] This investigation was sponsored by the Commission on Neurotropic Virus Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army, Washington, D. C. The laboratory work was carried out at The Rockefeller Institute for Medical Research, Princeton, N. J., and we wish to express our indebtedness to Dr. H. S. Gasser and Dr. C. TenBroeck for the facilities which were provided us. The participation of the inmates of the New Jersey State Prison at Trenton, N. J., who volunteered, without any offer of reward, to serve as subjects in these experiments, and the cooperation of the late Commissioner William J. Ellis, of the Department of Institutions and Agencies of the State of New Jersey, in providing facilities for this work at the N. J. State Prison, are hereby gratefully acknowledged.

[2] The specimens from Hawaii were provided on March 6, 1944, by Lt. Col. Clarence S. Moran, M.C., commanding the Central Pacific Area Laboratory, and those from New Guinea by Lt. Col. Cornelius B. Philip, Sn.C., of the U.S.A. Typhus Commission.

[3] Our associate, Capt. Wm. G. Jahnes, Jr., Sn.C., raised and took care of the mosquitoes used in these investigations.

[4] A. B. Sabin. Unpublished observations on dengue.

[5] We are indebted to Dr. Wendell M. Stanley for his help in the ultracentrifugation work.

[6] J. E. Dinger and E. P. Snijders, *Arch. f. Schiffs u. Tropenhyg.*, 35: 498, 1931.

has been more often in the range of 2 weeks, and the incidence of manifest paralysis with fatal termination has increased especially since the 9th passage. However, only about 60 to 75 per cent. of the inoculated mice exhibited clinical signs of the infection as late as the 13th passage. In the 14th passage, 90 per cent. of 20 approximately 3-weeks-old mice developed distinct central nervous system (C.N.S.) signs, but only 1 of 10 six-weeks-old mice exhibited the same signs, indicating the importance of using young mice for routine passage. In the 15th passage all 10 approximately 3-weeks-old Swiss mice developed C.N.S. signs, but of 10 mice of about the same age of an albino strain bred for many years at the Rockefeller Institute at Princeton, N. J., only 1 was affected. Simultaneous tests with 5th passage virus on other breeds of mice (6 varieties obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, and a group of brother-sister inbred Swiss albino mice for which we are indebted to Dr. Clara Lynch, of the Rockefeller Institute in New York) have indicated that different "strains" of mice may vary in their capacity to develop clinical signs of infection. The "dba" (dilute brown non-agouti) strain obtained from Bar Harbor appeared to be the most susceptible.

The mouse-passaged dengue virus was not pathogenic for cotton rats and guinea pigs (passage V and XIII) or for hamsters and rabbits (passage V). This limited host range may be used to differentiate it from two groups of viruses occasionally carried spontaneously by mice, *i.e.*, lymphocytic choriomeningitis and the encephalomyelitis viruses isolated by Theiler. A preliminary pathological study of the central nervous system of mice infected with dengue virus indicates that the attack is not on the meninges or the ependymal structures; it would appear that the neurones are affected, but the actual number of neuronal and infiltrative lesions is relatively small even in mice that are completely paralyzed or die of the infection.

The following attempts have been made to determine whether or not serial propagation of dengue virus in mice may be initiated *without* using concentrated, ultracentrifuged virus. In addition to inoculating undiluted, infectious human sera we also injected the 1:10, 1:100, and 1:1000 dilutions in 10 per cent. normal human serum-Tyrode's solution, and the best results were obtained with the 1:100 dilution.[7] The data seemed to suggest that the sera may perhaps contain an "inhibitor" as well as virus, and that either concentration of the virus by centrifugation or dilution of the "inhibitor" may achieve a suitable ratio between the two. Although three consecutive passages have been accomplished thus far in a series initiated with one diluted serum, the same difficulties were encountered in adaptation of the virus to mice, *i.e.*, initial low incidence of clinical signs, long incubation periods, necessity of using large numbers of young mice and persistence in repeating negative passages. When a suspension of dengue-infected *Aëdes aegypti* mosquitoes was injected intracerebrally in mice only 2 out of 20 exhibited clinical signs of infection, and a second passage did not yield any better results.

Thus far, in addition to the Hawaii strain of dengue virus, it has been possible to obtain positive serial passage in mice also with the New Guinea "A" strain which is immunologically identical with it.[4] The New Guinea "B," "C" and "D" strains, which, while related to the Hawaii strain, are, nevertheless, immunologically distinct,[4] thus far have yielded negative results, but further attempts to adapt them to mice are still in progress.

The decision to test the mouse-passaged virus in human beings was not made until 5 passages in mice (requiring about 3 months) had indicated that we were dealing with an agent unlike any other hitherto described. Sixteen human volunteers have now been inoculatd with various passages of the mouse-adapted virus—pool of 2nd and 3rd (1), 4th and 5th (1), 5th (3), 6th (2), 7th (3), 9th (2), 10th (4). Like the original virus in human serum,[4] the mouse-adapted virus produced skin lesions at the sites of intracutaneous injection, and 6 to 9 days after inoculation systemic manifestations, including fever, very marked maculo-papular and petechial rash, leukopenia and enlargement of certain lymph nodes. Immunity to infection with the regular dengue virus invariably followed. Although it was evident from the beginning that the virus had undergone a change even as a result of 2 serial passages in mice, fairly severe types of experimental dengue infection were produced in some of the volunteers inoculated with virus from the first 6 passages. 0.2 cc of a 1:1000 dilution of centrifuged brain and cord suspension from a pool of 5th-passage mice was sufficient to produce the disease in man and there were indications from skin reactions that a smaller dose might also have been infective. The virus was demonstrated in the blood of the inoculated human volunteers and transmission by *Aëdes aegypti* was accomplished, although relatively large numbers of mosquitoes and an extrinsic incubation period of 3 weeks or longer (10 to 14 days is usual for the unmodified virus) seems to have been required.

Tests with the 7th, 9th and 10th passage material

[7] The diluted sera were inoculated at the suggestion of Dr. Max Theiler, of the International Health Division of The Rockefeller Foundation, in view of his observations on the primary transmission of certain strains of yellow fever virus to mice.

on 9 volunteers indicated that the mouse-adapted dengue virus had undergone a very extensive modification in its pathogenicity for human beings. The intracutaneous or subcutaneous injection of 0.2 cc of a 5 per cent. centrifuged brain and cord suspension gave rise either to no systemic symptoms or after an incubation period of 8 to 10 days to reactions not exceeding in severity those following typhoid vaccination, *i.e.*, fever with or without headache and malaise for 24 hours or less. A marked and extensive maculo-papular eruption and terminally also petechiae on the feet and ankles were the rule. However, when this dose of mouse-adapted dengue virus was given simultaneously, or mixed, with the regular U. S. Army dose of yellow fever vaccine, the rash was reduced to a small number of faint macules and the petechiae were entirely suppressed. This combination was tried because one of us (A.B.S.) had previously observed an interference phenomenon between yellow fever vaccine and the regular, unmodified dengue virus in human beings.[4] It has, furthermore, been demonstrated that the virus present in the blood at the onset of the rash in the volunteers inoculated with 7th passage virus was of the modified type, and that *Aëdes aegypti* feeding during that period became infected with difficulty since large numbers of mosquitoes and an extrinsic incubation of more than 3 weeks were required to transmit the virus. However, the virus that these mosquitoes transmitted was also of the modified type, since rash without fever or significant symptoms and the leukocyte changes seen in dengue were the only reactions observed in the bitten individual. The 9 volunteers who were inoculated with the 7th, 9th or 10th mouse-passage dengue virus, either alone or in combination with yellow fever vaccine, and 1 volunteer who was bitten by mosquitoes carrying the modified virus, were all found to be immune when they were exposed to the bites of *Aëdes aegypti* mosquitoes of proved infectivity at intervals of 12 days (2 men), 21 days (5 men), 24 days (1 man) and 28 days (2 men) after inoculation. Four volunteers, who served as controls for these tests and were bitten by mosquitoes from the same lots, developed typically severe unmodified dengue. The volunteers, who received the combination of dengue and yellow fever vaccine, also developed neutralizing antibodies for the yellow fever virus.[8]

SUMMARY

It has been demonstrated that dengue virus can be propagated by intracerebral inoculation in mice. Although initial adaptation to the mouse is a tedious and difficult process, 16 consecutive passages have been achieved already in one series and further passages are in progress. The virus propagated in mice produced dengue in human volunters, but was not pathogenic for cotton rats, hamsters, guinea pigs or rabbits. Although it was evident that even after 2 serial passages in mice the virus produced a modified type of disease in human beings, tests with the 7th, 9th and 10th passage material indicated that the modification had become so marked that it could be used as a vaccine for the production of immunity against dengue.

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[8] We are indebted to Dr. Max Theiler, of the International Health Division of The Rockefeller Foundation, for carrying out the yellow fever neutralization tests on these sera.

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF 2,4-DICHLOROPHENOXYACETIC ACID

THE specificity of 2,4-dichlorophenoxyacetic acid in bringing about the death of certain weeds[1, 2] and the well-known bacteriostatic properties of some other aromatic compounds suggest that 2,4-dichlorophenoxyacetic acid might affect the growth of some lower organisms such as fungi and bacteria.

It was observed that open test-tubes of potato-dextrose agar[3] containing 0.1 per cent. 2,4-dichlorophenoxyacetic acid dissolved in 0.5 per cent. Carbowax 1500[4] remained sterile for 30 days, while similar tubes containing no 2,4-dichlorophenoxyacetic acid became contaminated with bacteria and fungi.

In a subsequent experiment two lots of potato-dextrose agar were prepared, one containing 0.1 per cent. of 2,4-dichlorophenoxyacetic acid and 0.5 per cent. Carbowax 1500 and the other containing 0.5 per cent. Carbowax only. The reaction was adjusted with N/1 NaOH to give pH values of 5, 6, 7 and 8, respectively, in aliquots of each of the two series. All manipulations involving the media were made in the open laboratory. The media and utensils used were not sterilized and no attempt was made to avoid contamination. Five Petri dishes were used for each medium, 15 ml being used for each dish. After the media had solidified, spores of *Penicillium* sp. were dusted over the surface of all plates. The plates were then closed and incubated at 28° C. Within two days colonies of

[1] J. W. Mitchell, F. F. Davis and P. C. Marth, *Golfdom*, October, 1944.

[2] P. C. Marth and J. W. Mitchell, "2,4-Dichlorophenoxyacetic Acid as a Differential Herbicide," *Botanical Gazette*, 106: 224–232, 1944.

[3] 200 gms peeled potatoes, 20 gms dextrose, 15 gms agar, 1,000 ml water.

[4] J. W. Mitchell and C. L. Hamner, *Bot. Gaz.*, 105: 474–483, 1944.