According to this criterion, 37 out of the 72 treated cases were found belonging to A-type (31 typhoid cases, 6 paratyphoid cases). In these cases, the duration of the disease was shortened by 10 days as compared with the 50 control cases treated by the ordinary method.

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STUDIES ON DENGUE (RESUME)

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1. Animal Experiment.

Since the early work of Graham (1903), the experimental studies of dengue have been carried out entirely upon human beings, needless to say, owing to the lack of suitable animals. But, apparent infection in lower animals, especially mouse, was observed by the authors at the outbreak in Osaka and Kobe in 1942.

First of all we attempted to inoculate mice, but did not forget the use of the fertilized chicken eggs at the same time to make sure of isolating the virus. In some instances, the last method apparently proved helpful.

The blood was taken within 24 hours from the onset of fever and using its serum, we isolated 6 strains of virus from 8 materials, termed as Fukuda, Takaki, Kawasaki, Kimura, Warabioka and Okajima.

We first succeeded in the inoculation of animals by the use of especial young mice, following our own experiences in the isolation of Lymphogranuloma inguinale or measles-virus, in which susceptibility was found to be very marked in the young animals.
The use of especially young mice may, we believe, have been a main reason of our success in the isolation of the present virus. Intracerebral injection was used throughout the experiments, but from time to time other routes, such as subcutaneous or intraperitoneal injections were also tried.

Apparent infection of other animals such as young guinea pig, rabbit, rat and monkey, was also attained, when we used the mouse-virus.

General symptoms of infected mice:

Infection and incubation periods were, as a matter of course, inconstant in the early stage of isolation. The incubation period varied from several to 17 or 18 days. Even the longer incubation periods than 25 days were also seen, occasionally.

The tendency of adaptation, that is, the short and constant incubation period as usually seen in other fixed viruses, has been observed following passages through many generations of mice.

The virus-fixture, obtained after many passages is capable of killing the adult mice of 15–16 grams under apparent infection within 4 or 5 days.

Although there exists certain divergency in the symptoms according to the individual strains or grade of adaptation, general findings among the strains, nearly or completely adapted to the mouse, may be stated as follows:—

1. Brain symptoms.
   Symptoms set about in rapid cases after 2 or 3 days, in most cases 4 or 5 days and rarely 2 weeks. There first appears paralysis on legs, the mouse reeling along, losing balance and lying on their belly. Further, some animals shrink round their body, irritable, tremble with hair standing on end. And, when the sickness proceed, the mice roll about on floor and soon succumb (chronic cramp).

2. Swelling on legs.
   In some instances, the swelling appears on legs and when this symptom comes over the whole legs, the motion of mouse becoming very slow owing to the consequent paralysis, and it dies soon after.
   The swelling is caused readily by a certain strain of virus but no readily by others.
   At times, slight cyanosis appears on legs.

3. Bloody excrements or haematouria are seen at times.

4. Conjunctivitis is found rather often; the eyes are closed by the deposition of a lot of discharge.

5. Dyspnoea, asthenia, decrease of body-weight, etc.

Autopsy findings:

1. The brain is seen congested passably in many cases.

2. Congestion of lung (interstitial pneumonia) is seen in almost all cases, although the grade of which is so variable as to vary from the liver-like total affection to punctated purpura or beginning blush.

3. Often liver appears yellowic-white or its normal brick-colour faded (fatty
(4) In kidney oedematous swelling and discoloration as in the case of liver, is occasionally seen (glomerulum being injured).

(5) Suprarenal body is congested in about 50 per cent of cases.

(6) Certain pathologic change in heart (myocarditis).

(7) Spleen is mostly normal in appearance.

(8) Pustule or abscess on the organs, as lung, heart, liver, kidney, etc. (extensive infiltration composed mainly of leucocytic cells).

The pustular change is, however, found only in very rare cases.

Susceptibility of other animals to dengue:

Results of inoculation of the mouse-virus are as follows:

(1) Guinea pig is killed by the intracerebral injection and we are able to pass the infection through several generations.

(2) Also rat (house and albino) can be infected in the same way, if inoculated intracerebrally or subcutaneously.

(3) Rabbit dies if inoculated by intracerebral or intraocular route. But, we failed to transmit the virus in a series even by means of the intracerebral injection of the brain-tissues.

(4) Monkey, inoculated with virus subcutaneously, showed a febrile reaction of about 40°C, leucopenia (3000), gingivitis, etc.

It was found, however, that by means of the intracerebral inoculation, the same animal is infected more apparently and dies after 3 or 12 days' incubation period. Owing to the paralysis of legs the infected monkey appears really a “dandy” itself.

At the autopsy, slight pneumonia and fatty degeneration of liver, were detected. The pathologic change in the suprarenal gland or kidney was not so marked, in that, it resembled the pictures usually seen in the mice inoculated with virus, not yet fully adapted to the mouse. We were able to pass the infection through 5 generations.

2. Cultivation of Dengue-Virus In-Vitro.

a. Maitland-Maitland's method:

Dengue virus, which was passed through mice for 11 generations, was cultivated in a flask, containing Tyrode's solution and finely minced brain of chicken embryo (10—12 days' incubation) for 10 generations.

The same result was obtained also by means of minced whole embryo incubated 8 days.

Transmission of virus to the next flask, was performed suitably every 5 days. The pathogenicity of cultivated virus on mouse did not decrease by the prolonged in-vitro cultivations.

b. Goodpusture's method:

Although the lesions on the chorio-allantoic membrane characteristic to dengue as described by Shortt and others was hardly observed, this method found especially suitable in the cultivation of the present virus.
Furthermore, the method seemed to help the adaptation to mice as it was observed in the case of Fukuda, Warabioka and Okajima strains. Namely, the virus used to become so virulent as to kill the embryo within 4 or 5 days, when cultivated on chorio-allantoic membrane for 4 or 5 generations. Moreover, the virus virulent to the embryo, appeared also to infect the mouse more readily.

The chick embryo, inoculated with a large dose of virus dies within 4 days, accompanied by the haemorrhage or bloody spots upon the whole surface of the body.

The haemorrhagic change mentioned above seems to be more characteristic to dengue than the cloudy or whity spots on the membranes as pointed out by Shortt and others. So far, although a whity spot or turbidity on the membranes is seen also in the case of measles, but haemorrhage in the site of embryo is not so marked.

Two strains (Warabioka and Okajima) have hitherto been cultivated solely upon chorio-allantoic membranes for 99 generations (19/XI, 1944).

3. Particle Size of Dengue-Virus and Some Physical Influences on It.

(1) Diameter of dengue-virus (mouse-virus) was estimated as 15—23 mμ (19 mμ in an average), by means of the ultrafiltration of Elford. The size precisely coincides with that of yellow fever-virus as actually demonstrated by Bauer.

(2) Dengue virus differs much from ordinary filterable viruses in its low resistance to the action of phenol; the virus, suspended in 0.5% phenol solution shows certain attenuation after 24 hours, and is almost completely inactivated after 5 days, all at room-temperature of 23 or 27°C.

The same virus, when kept in a cold-room (6—8°C), maintains its original titre for 48 hours, but certain drop in titre is observed after 5 days. The control virus, without phenol, was fully virulent even after 7 days.

(3) The virus is partially inactivated at 56°C for 5 min. and completely destroyed after 30 minutes at the same temperature.

(4) The virus is inactivated by the exposure to supersonic waves.

(5) Ultraviolet-light also inactivate the virus.

(6) The virus is affected by X-rays. Although the inactivation is not yet marked at 25,000 r., it is highly damaged by 50,000 r. and completely by 100,000 r.

The completely inactivated virus thus obtained was found to have a very potent antigenic properties.


Sera taken from dengue patient have certain amount of neutralizing antibody, but it cannot be said to be sufficient for practical purposes.

The immune serum, which was prepared by the repeated injections of the infected mouse brain into rabbits, proved that it contained very large amount of antibodies.

The authors herewith, could establish not only the identification of many strains but also detect the probability of the prophylaxis and therapy of dengue by means of sera. Furthermore, the side-by-side immunization was proved useful in the immunization of animals.
Results of application of immune sera upon patients:

Encouraged by the experimental success mentioned above, the authors have prepared the immune serum using goats and a horse. We had an opportunity of testing its effect upon dengue patients at the second epidemic of Osaka (1943). So far we could try our immune sera upon 12 patients, together with 2 patients, which received normal horse serum as control.

The results were as follows:

Certain therapeutic effect was observed if it was used within 72 hours after the onset of fever.

It appeared especially effective when it was used at the early stage and in larger doses, as a matter of course.

The immune sera, however, seemed inert contrary to our expectation, to the fever, allowing the typical saddle-like fever in about 50 per cent of cases. But, notwithstanding the general conceptions that the pains suffered by the patients at the second rise of temperature is more severe, the patients treated with immune sera, were found to be much more relaxed and feeling merely feverish against the high temperatures.

If we calculate a “level of significance” after the method of direct probability of R.A. Fisher, estimating as “effective”, when the recovery of appetite and the subsiding of the general pain were evident before 3rd day of disease, it being smaller than 0.05, sufficiently indicates the more effectiveness of immune sera than the normal horse serum.

To sum up, if immune serum, was applied to patients appropriately, although it by no means appreciably affect the fever-curve, it evidently shorten the duration of disease, that is to say, it hasten the recovery of appetite, decrease the head-ache and other pains.

Further, leucopenia is once reversed to normal although for a short duration of time. Normal serum exerts no effect on the leucocyte counts and general pains.

Serum therapy was repeated at Batavia and Bandong in 1944 to obtain the similar results. Namely, leucocyte counts returned once to normal, followed by the betterment of appetite and recovery of asthenic states.

5. The pathogenicity of Mouse-Brain Virus on Man and Its Immunogenic Properties.

Eight volunteers including the authors were inoculated with 2 representative strains, one already fixed to mouse (Fukuda) and the other still on the way to adaptation (Kimura or Warabioka). Heart-blood of infected mouse in varying doses of 0.05cc to 2.5cc was injected intradermally to the above volunteers:

Out of these 8 volunteers, from case 1 to 6 contracted dengue very atypically and case 7 almost typically. In the former 6 cases, temperature was found very low in general, including afebrile cases (cases 5 and 6).

The fact that the rash, if appeared, did localized at the site of injection, is noteworthy.

Case 8, which was inoculated with 2.5cc amount of blood of mouse infected with
Kimura-strain, developed a typical dengue.

Further, in view of the general pains, the pathogenicity on man appears very closely connected with the virulence to the mouse or adaptation-grade of the strains used.

For instances, the symptom is apparently mild when volunteers were inoculated with Fukuda strain, which is completely adapted to mouse.

On the contrary, it was found that the pathogenicity upon human being is still sufficiently remaining, in the case of 2 other strains (Kimura and Warabioka), which as yet not completely fixed to mice.

Next, as regards the leucopenia, in every cases with the exception of case 8, it was not so marked, but increase in number of the young neutrophilic cells, or the so-called "shift to the left" and plasma cells were observed constantly.

Among all blood change, however, the rapid increase of the eosinophilic cells, as 4% at the lowest and 14% at the highest; 400—1,000 cells were counted absolutely—was thought as the most characteristic.

To sum up, the pathogenicity of our mouse-virus upon human beings becomes low in an inverse proportion to its adaptation-grade on mouse.

Further, regarding the blood picture, especially, in the point of eosinophiles, our mouse-virus apparently differs from the ordinary dengue.

However, 2 out of 12 cases which were investigated by the authors at Singapore, eosinophilie was very marked at the beginning of the disease, though, and it was especially marked in a reinfection case.

Immunity:

After 10 or 32 days, these experimentally induced dengue cases mentioned above were reinoculated with Kimura-strain which is still virulent for man as already described elsewhere in this paper. Two out of five persons tested were found to be refractory, and the remaining 3 persons contracted dengue, though mildly, but all found to be afebrile and showed no rash. A control (case No. 7) inoculated with the same test virus fell ill with dengue with fever of 38.1°C, rash, headache, and lumbago, etc.

With these findings, it was confirmed that the immunity develops through the infection and that it takes more than a month to reach the height.

To sum up, our mouse-virus is apparently attenuated and yet certain amount of immunity is caused by its infection. Our prophylactic measures are now going to develop based on this finding.

Several persons have hitherto been vaccinated at their departure to the southern countries.

A party including 2 girls went to the southern countries and the members fell ill with dengue one by one. A girl also contracted dengue at Macassal 4 months after the vaccination under a very slight fever of about 37.5°C. Rash was typical, sufficient to know that she was really attacked by dengue. Another girl and a gentleman have not at yet been attacked by dengue.
6. On the Result of Prophylactic Vaccination against Dengue performed in Java.

Okajima or Fukuda strain was used in the preparation of vaccine.

Brain of young guinea pig or monkey, infected with the above mouse-virus, being ground in a mortar, added with normal saline solution to make it 20% emulsion, centrifuged, and the supernatant fluid thus obtained, was used as the prophylactic vaccine.

In addition, a formalized vaccine was prepared adding the formalin in 0.15% to the above vaccine and incubating at 37°C for 24 hours.

The vaccines were injected subcutaneously in one or 2 doses in this case.

The reactions were apparently milder as compared with the previous experiences, when the virus-material (mouse-blood) was inserted intradermally.

Namely, almost no recognizable reaction was found at the 1 dose, but at the injection in 2 doses, 7 or 10 days after the first injection, localized red area appeared at the site of injection, and almost all people complained of passably severe itching. This local reaction may be said to be the main complaint of many vaccinants.

In some individuals, however, temporary head-ache, heavy feeling in the head, fatigue or swelling of the axillary lymph gland were observed, but all these being of transitory nature nobody became incapable of their daily fatigue duty.

Experimental:

(1) Results of vaccination performed at Batavia.

Infected monkey-brain of 3rd generation was used as vaccine, in an amount of 2 cc. This being done in April 21, 1944, the state of the spontaneous infection was observed to find out no case of infection among 37 persons of A-group, during 3 months after the vaccination. And one typical dengue was found from 5 non-vaccinated persons.

Next, among B-group (24 vaccinants) one contracted dengue at Surabaya just 2 weeks after vaccination. This, however, may be assumed as the contraction during the immunity has not yet fully developed.

(2) Results of vaccination made at Timahi near Bandong.

Sixteen volunteers from a Japanese troop were vaccinated with the vaccine prepared by the guinea pig-brain, infected with Fukuda (or Okajima) strain.

Nine volunteers were vaccinated with living virus-preparation; 7 volunteers were with formalized vaccine.

First dose (10/IV, 1944) 2 or 3cc, 2nd dose (15/IV, 1944) 3cc, and after 38 days (18/V, 1944), the immunity test was performed using an active virus taken from a patient (Furukawa). Titre of the patient serum being found as 10^{-3} by the intradermal injection, its 10^{-1}cc was injected to the 16 vaccinated volunteers, but all with only one exceptional case, who was vaccinated with the living vaccine, contracted typical dengue.

(3) Results of vaccination of A-group of Bandong.

Vaccine prepared from guinea pig-brain (Okajima-strain) was used in this case.

Ordinary vaccine was used in 1cc at 1st and 2cc at 2nd injections. Formalized vaccine was used in 2cc at 1st and 3cc at 2nd dose.
After 42 days, 10^{-2}cc of the same patient serum (Furukawa) was injected to the above vaccinants. Two volunteers were inoculated with the same test-virus at the same time.

As the outcome of this susceptibility test, any protection was difficult to detect among persons, vaccinated with the formalized vaccine.

And, of the 3 persons, who have been vaccinated with living-vaccine, one was found as completely refractory to dengue and the remaining 2 contracted dengue, but so mild as to be confined to bed only for 1.5 and 3 days, respectively.

Two freshly infected volunteers to serve as control and 3 previously vaccinated with formalized vaccine, altogether contracted typical dengue and were confined to their bed for 6 to 10 days.

Preeminence of living-vaccine was thus demonstrated.

(4) Results of B-group of Bandong.

Twenty volunteers were vaccinated. The vaccine was prepared by means of the monkey brain (5th generation), infected with Fukuda-strain.

First dose in 2cc (4/V, 1944) 2nd dose in 3cc (10/V, 1944). And on May 31, that is, 27 days after the first vaccination, 10 vaccinants tested on their resistance to dengue; 3 were injected with 10^{-2}cc and the remaining 7 injected with 10^{-3}cc respectively, of the same test virus as used in the preceding experiments. As the result, among 3, inoculated with 10^{-2}cc, one, severely and 2, slightly contracted dengue and among 7, which were infected with 10^{-3}cc of test-virus, only one contracted dengue, mildly.

Briefly:

1. Protection against dengue is attained by means of the attenuated mouse-virus.
2. More than a month seems to be necessary for the immunity produced by the vaccination to reach its maximum.
3. It was confirmed that we can protect against the infection of about 100 times of the minimal infecting dose of a active dengue-virus.
4. Presumably protection against dengue by means of the formalized vaccine, cannot be expected.

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EXPERIMENTAL STUDY ON MEASLES VIRUS — ARAKAWA

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In a previous communication, Yaoi and Arakawa (1) have described on inoculation experiment of measles virus upon mice by intraecerebral injection.

Recently, the author has succeeded in the fixation of the same virus, by using the blood of a patient(2).

The virus causes the same symptoms in mice, and became “fixed” after 4 passages through mice: One of the 10 first generation mice was killed under doubtful symptoms and obtained the 2nd generation in which, 3 of 10 mice became ill within 3 to 14 days and died in 10 to 30 days.

In the 3rd generation, 5 of 5 animals fell ill on the 3rd to 11th days. In all the succeeding generations all mice inoculated contracted the disease and died on about 5th day, thus, the fixed virus was obtained.

Infected mice are very irritable, on being touched they would cry “Chi-Chi” and lift their fore legs.

They assume an attitude as if to stand erect elevating half the body. Some times they bite experimenter’s finger. Soon after, the mouse stretches forth the legs and tremble, and after 2 or 3 hours at the most, bends its body backwards like a bow and dies in rigidity.