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DENGUE FEVER IN AUSTRALIA.

ITS HISTORY AND CLINICAL COURSE, ITS EXPERIMENTAL TRANSMISSION BY *STEGOMYIA FASCIATA*, AND THE RESULTS OF INOCULATION AND OTHER EXPERIMENTS.

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(With IX Charts.)

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INTRODUCTION.

Epidemic Dengue first reached Australia early in 1885. In the same year, according to Castellani and Chalmers in their *Manual of Tropical Medicine*, it reached the Fiji Islands, "to which it was conveyed by a European suffering from the complaint." As later on in the same year a record occurs of cases of dengue fever on a steamer which arrived in Sydney from Fiji and Noumea, it is possible that the disease reached Australia from Fiji. Since this date, from time to time very extensive epidemics of dengue have occurred in Queensland, sometimes extending to the northern coastal towns of New South Wales.

A careful comparison of previous *clinical* descriptions of the epidemic disease known as dengue in Australia, with the description of the disease compiled from various sources as given in Castellani and Chalmers (*loc. cit.*), does not reveal anything tangible to suggest that more than one disease has, up to the present, been comprised under the term "dengue fever." The only important clinical difference appears to be that in the Australian disease, though the pulse varies more or less with the temperature, it is nevertheless relatively slow, and sometimes absolutely so[1].

Elsewhere in this report, chiefly as a result of our investigations, will be discussed the question as to whether or not under the broad term "dengue" it is possible that more that one distinct entity has hitherto been included.

Origin of these experiments.

In March, 1916, an extensive epidemic of dengue, then prevalent in Queensland, reached some of the north coast towns of New South Wales. The incidence on the population was exceedingly heavy, and business was greatly disorganised in consequence. As the experiments into its means of spread in Australia hitherto carried out had been few and inconclusive, it was considered advisable to visit the area affected and collect material there for further study of the disease. It was recognised that if infective material could be conveyed to Sydney, a town in which indigenous cases of the disease have never been known to arise, results might be obtained which would be free from fallacies attendant on experiments conducted in the epidemic area. At the beginning of April one of us, therefore, with an assistant, paid a visit to Mirwillumbah.

[1] A discussion of the attempted differentiation between Dengue and Pseudo-Dengue with special reference to the views of Rogers and Grall will be found in the recent monograph of G. Sticker, *Dengue und andere endemische Küstenfieber*, pp. 76, Wien, 1914. This work supplies an extensive bibliography of the literature, both clinical, epidemiological and experimental. (Editorial note.)

He found that though the chief incidence of the disease had passed, there were still numerous cases, and that two species of mosquitoes were very abundant in the town, namely, *Culex fatigans* and *Stegomyia fasciata*. He collected a number of both species of these mosquitoes, more especially from houses in which cases of dengue had occurred and preferably in the actual rooms of patients then ill with the disease. In addition he withdrew specimens of blood from patients suffering from the disease, some of them being allowed to clot and some being received in citrated normal saline solution. The materials thus obtained were brought back to Sydney.

At this early stage of the investigations, the following main objects were held in view:

1. To try to transmit the disease to human volunteers by means of one or other of the two species of mosquitoes which had been captured in the epidemic area.

2. To attempt to establish by inoculation of material from the blood a strain of the disease for further study as to the incubation period, symptoms and signs, immunity, etc.

With the mosquitoes in the first instance conveyed to Sydney, amongst which there had been a heavy mortality, successful transmission of the disease was not achieved. However, by the inoculation of materials obtained from the cases in the epidemic area and from the blood of a patient who had contracted the disease in the epidemic area and had reached Sydney before he had recovered, and from the blood of one of us who had made the journey and who had contracted the disease in the epidemic area and had developed it after his return to Sydney, strains of the disease were successfully established in Sydney. Though unfortunately in the early cases some of the volunteers received inoculations of blood from two different sources, thereby obscuring certain data in connection with their cases, the main point attempted at this stage was achieved, namely, the establishment in human beings of strains of the disease by inoculation. The bulk of the experiments detailed later consisted of sub-inoculations from these primary inoculation cases. It has been considered advisable to tabulate in full, in the shape of an appendix, each individual in chronological sequence on whom experiments were made. Under various headings, the results of these experiments are discussed as a whole with the object of ascertaining what information of value in connection with the disease and indirectly with other similar diseases can be reasonably deduced from the results obtained.

The failure to transmit the disease by the first batch of mosquitoes brought down from the epidemic area led to another of us with an assistant visiting in May the adjacent town of Mullumbimby then suffering heavily from the epidemic. A further considerable number of *Culex fatigans* and *Stegomyia fasciata* were brought back to Sydney and the typical disease was conveyed by the bites of the batch of *Stegomyia fasciata* to four volunteers in Sydney, thus establishing conclusively the rôle that this mosquito can play in the spread of the disease.

I. THE HISTORY OF DENGUE FEVER IN AUSTRALIA WITH A SHORT SUMMARY OF THE CLINICAL DESCRIPTIONS OF PREVIOUS EPIDEMICS[1].

The first reference to the occurrence of cases of this disease in Australia appeared in the *Aust. Med. Journ.* of 1873 (XVIII. 160) in the statement that eight cases of dengue, contracted at Mauritius, had occurred on board the ship "Charles Auguste" during the last few weeks of May. (Melbourne.)

In 1885 a note is found in the *Australasian Medical Gazette* (April, 1885, p. 177) to the effect that the disease was prevalent in Rockhampton (Queensland) to an alarming extent. In the same year (*Aust. Med. Gaz.* September, 1885, p. 310) five cases of dengue fever are reported as being discovered on the A.S.N. Co.'s steamer "Gunga" which arrived in Sydney from Fiji and Noumea on August 17th.

In 1886 Dr J. A. Weber, of Natimuk, Victoria (*Aust. Med. Journ.* July, VIII. 291) reported an outbreak which was thought to be typhoid fever when the cases were first taken to the hospital, but which, after consultation with the Board of Health, was definitely considered to be dengue. He had seen about thirty-two cases during the previous four months. The incubation period was not discovered. There was no prodromal stage. Quite suddenly the patient was attacked with severe pains all over the body as if the bones were broken ("Break-bone fever"); there were severe headaches, and a most characteristic pain in the eyeballs, increased greatly on slight pressure. The temperature was 103° F., seldom higher, 104° being the highest recorded. The face was red and swollen, and there was also some slight swelling of the joints, and

[1] This section was contributed by one of us (J. B. C.) to the *Third Report of the Govt. Bureau of Microbiology* dealing with the work performed during the year 1912, Sydney, New South Wales, Govt. Printer, 1914. As this publication is inaccessible to many readers and the subject-matter is so applicable to the present Report, it is reproduced here in full.

frequently an erythematous eruption all over the body. There were also constipation and loss of appetite.

The symptoms gradually subsided in about three days. Relapses were frequent, usually about the seventh day, but were unaccompanied by redness or swelling over the face or body. Without complication, patients recovered at the end of eight days. Redness and swelling of the face were constant in all the cases, whilst erythema and swelling of the joints were only transitory. There were three cases of relapses, and one in which premature labour developed.

In 1894 the disease was prevalent in Thursday Island, nearly every male and the majority of the females suffering from it (*Aust. Med. Gaz.* July, 1894, p. 252). In 1895 it was very prevalent in Townsville (*Aust. Med. Gaz.* February, 1895, p. 79). In 1897 an extensive epidemic occurred in Queensland, which produced a discussion before the Medical Society of Queensland on 4th May (*Aust. Med. Gaz.* May, 1897, p. 235). In this discussion, Dr Thomson described the complaint as follows:

"It consisted of a febricula combined with an erythema, many cases being of very mild character. In some, however, the temperature reached 103° or 104° F. but this was always followed by a rapid defervescence. Pains were sometimes complained of in the muscles and joints. In nearly every case there was a cutaneous eruption, sometimes of a measly character; sometimes an erythema; and usually of a patchy nature. In some cases it was only visible on the thighs. Desquamation did not occur, and no serious results followed. The patients were left very weak, and convalescence was prolonged out of all proportion to the severity of the attack. In a few cases catarrhal symptoms, resembling those of influenza, were present. The disease was certainly infectious."

Dr Hopkins was of opinion that the disease was very like influenza, and that it did not correspond to the symptoms of dengue as given in the text-books.

Other speakers referred to the rash being commoner on the forearms —in one case only on the palms of the hands—and sometimes of a type intermediate in character between that of scarlet fever and that of measles. One observer had occasionally seen some desquamation.

Dr Halford said that the Brisbane Hospital had been inundated with cases; that the peculiarity of the rash was its multiformity, being sometimes macular, papular, diffuse, or punctiform; that some cases had a very slow pulse (36 to 40); that some had a rash without any malaise; and that half the cases shewed some increase of splenic dulness.

Dr Turner was of the opinion that only a very few cases had symptoms resembling somewhat those of dengue fever, but that the great majority of them shewed no resemblance beyond the presence of a rash. He said that the term "breakbone fever" could not be applied to the cases he had seen, and that it was impossible to identify the epidemic as that described in books as dengue, and these descriptions were misleading.

Dr C. S. Hawkes, of Rockhampton (Queensland), in a paper in the *Inter. Med. Journ.* (1897, II. 450) describes cases met with in the epidemic of Central Queensland in the early part of 1897. In one case—that of a patient who lived in a neighbouring township and on one occasion visited Rockhampton and returned, no other cases arising—the incubation period was definitely ascertained to be three days. The disease itself was very variable. The prodromal symptoms were sometimes absent; sometimes consisted of malaise and rise of temperature to 99° or 100° F. over a period of from twenty-four hours to fourteen days. The onset was usually sudden, with severe headache, often shivering, but rarely a well-marked rigor; then followed aching of the limbs, backache, prostration in severe cases, and the initial rash or a deep flushing of the face, with a hot and dry skin. The course of an ordinary attack is given as follows: "The pains and fever increase during the first twenty-four hours, aching being felt in all the limbs, more in the neighbourhood of or in the joints, though patients find it very hard to localise their pains; backache is always present across the lumbar region, headache all over the head, often some pain in the eyes, which feel sore when moved; pain is also felt across the upper part of the abdomen, vomiting increases it somewhat, but it is often severe when none is present; vomiting more often comes on about the third or fourth day, but may in some cases be present from the first; the tongue coats with a whitish fur, the breath becomes offensive, and the initial rash, if present, may be noticed.

During the second day there is usually some remission of symptoms, if not of temperature, though there is occasionally a drop of two or three degrees about this time; the patient feels more comfortable, even if the temperature has not fallen; this remission does not last long, the temperature, if it has fallen, soon rises again, the pains return with greater intensity than before, retching and vomiting are present, with great dislike for food, often with restlessness and insomnia, these symptoms persisting with little variation till the crisis takes place at the end of the fifth day, or a little later.

In severe cases the crisis may be well marked, with vomiting,

diarrhoea, profuse sweating, bleeding from the nose and, more rarely, from the uterus, and occasionally delirium or, rarely, excessive drowsiness. As a rule, during the last day, the terminal rash appears on the body and limbs."

Hawkes describes fully abortive attacks; ordinary attacks ending in about five days and with temperatures ranging in severe cases to 104°, 106°, and even occasionally to 107° F.; cases lasting for seven or eight days and ending by lysis and usually less severe than the ordinary cases; and a type in which the fever is prolonged over several weeks or recurs at irregular intervals. The fall of temperature on the second day was not noticeable in this particular outbreak. Of other points noted may be mentioned a slow pulse-rate, in some cases at the beginning of the attack and during convalescence; the muscular nature of the pains (*e.g.* those of the eyeball); occasional severe and persistent vomiting; the rarity of troublesome salivation; "black vomit" in one case; the frequency of nose-bleeding at or near the crisis; insomnia and marked mental hebetude, and sometimes mental aberration, and even delusions of sight by day in the absence of delirium. Enlargement of the glands was uncommon, but in a few instances the posterior cervical glands and those over the mastoid felt a ittle increased in size. As sequelae, persistent pains in the limbs and the upper part of the abdomen and aching in the orbits were not uncommon. Some cases shewed dyspepsia, with dislike for food and disorders of taste, such as all food seeming bitter. In one case this bitterness was associated with a disagreeable foetid odour. There were also several cases of pleurisy, a few of catarrhal jaundice, some with periostitis, and a number with boils. Slight mental hebetude was common, and in a few cases mixed aphasia with misplacing of words.

The initial rash during the first day of fever appeared as a deep flush on the face and irregular dark-coloured blotches on the chest, or as an erythematoid mottling of the chest, neck, and face. It disappeared in the course of a few hours.

The terminal rash was more marked, and was usually scarletiniform or morbilliform, appearing first on the upper part of the chest, and accompanied with a sub-cuticular mottling. A third type of rash was urticarial, sometimes appearing as a giant urticaria. With all forms there was some general irritation of the skin, itching of the hands and feet, and slight desquamation. Petechiae, sometimes extensive, occurred in some cases.

In 1898, Dr Eugen Hirschfeld, Honorary Physician to the Brisbane Hospital (*Intercol. Med. Journ.* 1898, III. 143) discusses in considerable

detail the two epidemics of dengue which had swept over Queensland from the north to the south during the previous twelve months. His paper should be consulted in the original.

This same epidemic of 1897 is discussed fully by Dr F. E. Hare, late of Charters Towers (*Aust. Med. Gaz.* March, 1898, p. 98), who collected valuable information from many sources. He points out that the epidemic commenced at Cooktown on 8th January, and reached Cairns on 8th February, and various other towns in North Queensland during March, Bowen not being attacked until 30th May; Herberton escaped. The whole paper is too long to quote here, but is well worthy of careful perusal. The information obtained is the result of forwarding circulars to most of the medical men in North Queensland, a number of whom replied.

A discussion on this paper appears in the same number of the *Aust. Med. Gazette*, p. 124, and a similar discussion which took place before the Medical Society of Queensland appears on page 130.

I find that this epidemic reached the northern districts of New South Wales in 1898 (*Ibid.* March, 1898, p. 135).

Dr J. Lockhardt Gibson, of Brisbane (*Ibid.* August, 1898, p. 339), describes a case of acute inflammatory glaucoma produced in a susceptible patient by dengue. The symptoms began on the second day of an acute attack of dengue.

In November, 1899, one death from dengue occurred in Brisbane (*Ibid.* January, 1900, p. 42), and two deaths are recorded in Brisbane in October, 1901 (*Ibid.* January, 1902, p. 46).

In the summer of 1904–5, a severe epidemic of dengue occurred in Queensland, on which a most complete report was compiled by a Committee appointed by the Queensland Branch of the British Medical Association. The members of this Committee were the President of the Branch (Dr Robertson) and Drs Thompson, Love, Turner and Wield. Their report appears in the *Aust. Med. Gaz.* (November, 1905, p. 616). As this report has been reprinted in the *Journ. Trop. Med.* 1905, VIII. 355, it will be unnecessary to do more than refer to it in this place.

Ninety-four cases were reported to have died from dengue in Brisbane out of a population of 125,672. The chief mortality occurred in March and April. If it could be assumed that half the population were attacked the mortality will stand as 1 in 668, if 75 per cent. were attacked 1 in 1003. An account is given of thirty-five fatal cases about which information was obtained. Some interesting information is appended

as to the spread of the disease from house to house in particular neighbourhoods.

This epidemic of 1904–5 reached Thursday Island in the latter part of January and the beginning of February (*Aust. Med. Gaz.* February, 1905, p. 91). Almost everyone was affected, but there was only one death—that of an infant. Several cases also occurred in the northern parts of New South Wales, as well as in Sydney (*Ibid.* April, 1905, p. 185). Dr Ashburton Thompson, in referring to this, said that the disease had many times been introduced into New South Wales in past years to his knowledge—the first time in 1886. It occurred when dengue was extremely prevalent in New Caledonia. It took place in August and September, when the weather was cool, but did not spread.

Dr T. L. Bancroft (*Ibid.* January, 1906, p. 17), in discussing the etiology of dengue, states that direct contact, *e.g.*, sleeping with a patient, will not give dengue. The intervention of an intermediate host is evident. As some people from the country visited friends in Brisbane by day and had got dengue, evidently *Culex fatigans* was not the agent, and probably *Stegomyia fasciata*—a day biter—was the culprit. Dr Bancroft experimented with the latter mosquito. Dengue occurred in one case after five days' incubation, the mosquito having been kept twelve days after biting. In another instance, a mosquito being kept ten days after biting, five days later a mild attack occurred. In two cases with a fifteen days' interval, and in one with a seventeen days' interval, nothing resulted.

Dr Fredk. Woolrabe, in a letter to the *Aust. Med. Gaz.* (February, 1906, p. 105), criticises Dr Bancroft's experiments, suggesting the possibility of natural infection in his positive cases.

Dr J. Lockhart Gibson (*Ibid.* May, 1906, p. 227, and Australasian Medical Congress, 1905, seventh session, p. 283), describes a case of keratitis in dengue. Three cases of keratitis dengue have come under his notice, and five cases of keratitis post-dengue. He says the dengue cases appeared to start as keratitis neuro-paralytica, but to this is added rapid infective ulceration of the affected cornea.

Dr R. A. O'Brien (*Ibid.* March, 1908, p. 121) states that clinical observation distinctly incriminates the *Stegomyia* (*Scutomyia*) *notoscripta*, the distribution of which was coincident with that of the dengue wave of three years ago. He says further that, since the wave of typical dengue, cases have been common in the north of Queensland resembling dengue in every detail, except for the absence of rash and the third-day remission. Recently typical cases had again begun to appear in those who had had

dengue three years ago, suggesting that the immunity was wearing out. These cases corresponded in blood picture to that described by Stitt and Balfour as existent in dengue, and in dengue only, viz., a leucopenia with a great initial drop in polymorphonuclears, as low as 35 per cent. in some cases that Dr O'Brien had, with a replacement by small lymphocytes, these in their turn giving place about the fourth or fifth day to large lymphocytes or large mononuclears.

As regards the occurrence of dengue in Western Australia, I have been informed by Dr J. H. Saunders that he has seen cases at Broome and Roeburne.

II. CLINICAL DESCRIPTION OF THE 1916 EPIDEMIC OF DENGUE FEVER ON THE NORTH COAST OF NEW SOUTH WALES.

The facts utilised in compiling this description were mostly obtained by observations and notes on cases seen by us on visits to the infected district, and by some observations on imported, mostly military, cases in Sydney. Thus our description is one largely of the symptomatology of the illness compiled from histories given us by patients, who, at the time, were suffering or had recently suffered from the disease. In particular we have little exact information as to the type of temperature or the pulse charts in the naturally occurring cases, and our descriptions of these are for the most part based on observations on our experimental mosquito-borne cases, or on what we have been told was the case by observers in the infected district. We have availed ourselves also of the excellent description by Goldsmid and Crosse[1] to which we refer the reader.

Onset. This, in the large majority of cases, is described as sudden. Frequently the patient will give the exact hour at which he was taken ill, and may narrate how before a certain time he was quite well, and that after an extremely short period, perhaps half-an-hour from the first symptom, he was prostrate with the disease. Out of thirty cases replying to questions as to the nature of the onset, twenty-five replied that it was sudden, three that it was gradual. In two cases the replies were doubtful.

The onset is usually accompanied by fever, headache, malaise and slight shivering, and to a greater or less extent by pains and aches, which are very characteristic in the typical cases. In certain cases the relation-

[1] Goldsmid and Crosse, Some notes on Dengue, *Med. Journ. of Australia*, May 6th, 1916, p. 377.

ship between the fever and other symptoms is less definite and the fever may precede or post-date the other symptoms.

Course. After the onset the disease runs a course lasting from a few days to a fortnight or more (four to seven days—Goldsmid and Crosse). There may be two periods of intensity of fever and symptoms, separated by a period, varying in length but usually only of a day or so, of comparative abeyance of fever and symptoms, during which the patient may regard himself as well. This double phase is, however, in our experience, by no means a constant phenomenon, and its absence cannot be regarded as militating against the diagnosis of dengue. Moreover, especially in mild cases, although a four-hour temperature chart may shew a distinct double phase variation, the symptoms and temperature do not always vary *pari passu*. In some cases there appears to be a tendency to relapse at a later period, but we have no very precise information on this point. In a typical case, after a sudden onset accompanied by a rapid rise of temperature, shivering and headache, and occasionally slight vomiting, the patient takes to bed with pains in the back and limbs and severe headache. He passes a very restless night and may be delirious. He finds it almost impossible to rest in any position. For the following day or so the headache and body pains are worse. The temperature soon falls, and this may be accompanied by sweating, and the patient gets up, not feeling very well, and with a dirty tongue and a residue of pains. One, two, or three days later the temperature goes up again and the symptoms return. The second attack lasts for one or two days, and then convalescence ensues. In the stage of onset there is usually an erythematous blushing of the skin, and later on, from the second to the seventh day, a more distinct rash frequently appears.

It will be best now to review *seriatim* the outstanding symptoms and signs of the disease as met with in the North Coast.

The temperature and pulse. We do not wish to discuss these fully at the present time as our investigations have not enabled us to take first-hand records of many natural cases. From the information we can gather, however, the double phase temperature is not constant, but inasmuch as few of the cases are in hospital where accurate records can be obtained we cannot dogmatise on this point.

Goldsmid and Crosse say "The temperature rose sharply and reached 101–103° F. During the course of the illness it remained high and did not undergo marked fluctuation. Not infrequently it reached 105° F. just before the termination of the fever. The fall was as rapid as the rise."

The pulse rate in natural cases has not come under our personal observation to any extent, but Goldsmid and Crosse confirm the results we obtained in our injection experiments. They say, "It (the pulse) was invariably slow in proportion to the temperature. A pulse rate of 75 to 90 was frequently associated with a temperature of 102° or 103° F. A more rapid pulse rate than 90 was rarely noted save just before the final fall of temperature."

The pulse rate and its relation to the temperature in experimental cases is discussed fully in a separate section.

The facies. The face soon assumes a very characteristic appearance, and in our experience this is one of the most useful signs of the disease. It looks red, swollen, hot and puffy. The eyes are usually somewhat injected, but there is not excessive lachrymation or any running at the nose. *Coryzal signs are notably absent* although it must not be forgotten that an ordinary "cold in the head" may coincide with an attack of dengue. Out of twenty-six cases questioned as to the occurrence of "running at the nose" only one described it as being present.

The facies of dengue has been described as resembling that of a person recovering from an alcoholic bout. It is also somewhat suggestive of the face in the early stage of measles but without the coryzal condition. The typical facies is most marked shortly after the onset, or, when this occurs, in the recrudescent period.

Headache. Headache is a practically constant phenomenon. Thus, out of twenty-six cases questioned all gave a history of headache. In some cases it was located as frontal; in others, as vertical or occipital; and quite frequently as "all over the head." Frontal headache is hard to distinguish from the characteristic eye pains, and the sufferer frequently refers to pains "at the back of the eyes." The intensity of the headache varies very much. In certain cases it appeared to be the principal cause of complaint, sometimes being described as "agonising," whilst in others it was referred to as slight.

Eye pains. The painful eyes are, in our opinion, quite one of the most characteristic single signs of dengue, and are almost always present in some degree. Sometimes the eyes are said to be aching severely and painful on movement, and we have seen cases where the whole head was turned rather than move the extremely sensitive eyes. In others, it is only by careful questioning that the presence of some pain or tenderness in the eye-balls or eye muscles is elicited.

Out of twenty-eight cases questioned, twenty-five described pains in the eyes and three denied their existence. Out of thirteen cases

questioned on the point, eleven said the eyes were painful to move, and two replied negatively.

Apparently the earlier symptom is pain in the eyes or in the "back of the eyes," easily confused with frontal headache. Later there is definite pain and tenderness apparently in the eyeballs, which is associated with pain on ocular movement and probably often with some photophobia.

Occasionally the eyes are described as "sore" which word may be used to refer to the irritation of slight conjunctival congestion, but conjunctival symptoms are never prominent and the adjective "sore" is probably frequently used to refer to the deeper-seated pains in the eyeballs.

General or body pains. These vary very much in degree, and are by some described as intense and agonising, and they may require the administration of morphine; in other cases they are described as "tired feelings," "gone in the knees," and "influenzal pains." In our experience the "break-bone" type of case is the exception, and the pains are, as a rule, not a very prominent feature. Sometimes their occurrence is denied in a particular case, or only elicited after careful questioning. Restlessness and inability to stop in one position is characteristic of some cases, and is probably closely linked to the body-pain symptoms. Out of thirty-two persons questioned, all described various degrees of *body pains* somewhere *in the spinal axis*, and out of twenty-six questioned, all described pains in the limbs. The back of the neck is a common seat of fairly severe pain (sixteen out of eighteen questioned). *Lumbo-sacral pain* is also common ("across the back"—fourteen out of fourteen questioned). Real *pains in the joints* appear to be uncommon apart from the general limb ache. Movement does not seem definitely to increase the pains, but when severe the patient generally takes to bed because of the pain and associated symptoms. The body pains of dengue are in our opinion not associated with any readily demonstrable lesion. No swelling, redness or tenderness were noted in any case. This is in striking contrast with the description by Osler, who refers to red, swollen and painful joints.

Abdominal pains. These were described by ten out of a series of sixteen cases questioned and are sometimes associated with diarrhoea. At other times they are apparently a "spreading round" from the lumbar and dorsal region of the back pain. Pain in the epigastric region associated with vomiting is spoken of by Goldsmid and Crosse as occurring in several cases.

The body pains gradually subside, but there is usually a recrudescence of the pains when the second phase occurs. After the febrile stage is over there is generally some tiredness or aching for several weeks in the spine or limbs, which, however, gradually passes off.

Other nervous symptoms occurring in the disease are giddiness, delirium, mental irritability, depression and sleeplessness.

Giddiness is a common feature at various times in the course of the disease (nineteen out of twenty-two questioned).

Delirium is not frequent, but we have seen cases where there was maniacal delirium for three nights after the onset, and many cases shew some mental wandering when the temperature is high. Delirium was mentioned as a symptom in six out of twenty-one cases questioned on the point.

Mental irritability is a striking feature of the disease, especially in the later stages.

Depression. The depression following the attack is one of the most marked features, and the patient may be actually incapable of concentration or serious mental effort for a week or so after the attack.

Sleeplessness is found at some time in nearly all cases.

Gastro-intestinal symptoms are not marked. There is, however, a *dirty tongue* which is rather characteristic, being furred at the back with a strawberry tip very like that seen in scarlet fever. The *fauces* are reddened—Goldsmid and Crosse note a fine stippling of the soft palate as an early characteristic sign—and there may be some sore throat. This is usually not marked. *Anorexia* is a feature in most cases (thirty out of thirty questioned). *Nausea* is fairly common (eighteen out of twenty-nine questioned), and *vomiting*, though not as a rule marked, is met with especially at the onset (thirteen out of twenty-nine questioned) and occasionally may be severe. Some cases suffer no disturbance of the bowels, but *diarrhoea* is present in a few cases (two out of twenty-nine). *Constipation* is not general (four out of eleven).

Shivering occurs commonly (twenty out of twenty-four cases questioned). It may occur with the onset and during the febrile stage, but rigors are the exception.

The skin eruptions. According to Goldsmid and Crosse the preliminary and terminal rashes were well marked in cases seen by them. They note, however, that the preliminary rash could easily be overlooked. They describe this as a "fine punctiform rash usually found over points of friction....It appeared and disappeared very suddenly.... A fine stippling of the soft palate was often the only rash present when

the patients were first seen." They describe the terminal rash as "polymorphous," and as being present in nearly every case. "It was either papular or a dark red, blotchy rash, or an urticularia."

Our own experience probably covers a somewhat different type of case from that seen by Goldsmid and Crosse, as a great number of dengue sufferers seen by us had not consulted any medical man at all, and these were generally the mild cases which may shew an absence of certain symptoms or signs. Thus, while we agree in the main with them, we would modify their description in certain particulars. We do not think a rash is often entirely absent, but it is often so transitory or slight that unless the patient is under medical examination, and even then at times, it is easily overlooked. This applies not only to the preliminary but to the later rash. Our experimental cases bear this out. It will be seen that in several cases we were unable to make up our minds at all as to whether a rash was or was not present.

The distinction also between the prodromal and later rash is, in our opinion, not very valuable. Although in some cases it is possible to note definite skin eruptions at two periods separated by a period in which the rash is absent or not distinct, there are such great variations in the degree and type of the skin conditions of dengue that the distinction into two rashes is not of great value.

Early in the disease it is unusual to find a definite eruption though we have seen cases with well-marked measly rashes within forty-eight hours of the onset. A hypersensitiveness of the skin which tends to the production of blotchy erythema on points of pressure is an early sign and *tache cérébrale* is well-marked in most cases. The red congested condition of the face has been referred to before. In the early stage it is quite frequent for two observers to differ as to the presence of a rash. The more definite skin eruption is generally found later. Though it may be found well-marked from the second day, it may not be noticed till the fifth or seventh day. It presents somewhat variable characteristics and lasts from a day or so to (rarely) several weeks, and is usually followed by slight desquamation and sometimes by intense itching.

We have not sufficient data to describe accurately the distribution of the rash, but we have found it affecting almost any part of the trunk and limbs. It seems as a rule to be less distinct on the face which merely shews congestion. On the back, especially in the lumbar region, it is frequently very distinct and extends round to the abdomen where it is often less apparent. The legs and arms are frequently affected, as a rule the arms shewing more definite lesions. The hands are liable to

be affected, and bright pink spots, followed by intense itching and desquamation, are sometimes found on the palms.

The characteristic of the rash has been described by someone as its "want of characteristic." We think a good definition for the rash in many cases is "mid-way between that of measles and scarlet fever, but less definite." It is, as a rule, some form of a blotchy erythema, though especially in later stages the eruption does not completely fade on pressure. The size, shape and intensity of the blotchings to a great extent account for the differences in appearance. In most of the cases seen by us patches of red skin alternate with pale (normal) patches in a most irregular mottling. The red patches shew no definite point of maximum intensity, but at times the hair follicles are red and prominent giving a strawberry appearance to the red blotches. The red areas do not shew definite lines of demarcation from the adjacent normal skin. The blotchings vary in size but are usually not more than a quarter to half-an-inch square. On the legs of one patient, however, there were large irregular patches much larger than this and of a very bright pink. At the same time this case had a dull measly mottling on the trunk.

In some cases we have seen a very characteristic reddening and swelling of the elbows of a peculiar tint suggestive of a stain of eosin that has been partly washed out. This may be surrounded by a papular condition in the vicinity. Papular rashes have been rarely noted by us, but sometimes are seen on the feet or lower legs.

We have seen no urticarial cases, but these are described by Goldsmid and Crosse and others, and some patients have told us they had had this condition. They present another variation of the skin lesion.

Two other skin conditions should be noted here. In the North Coast district we have seen several cases of a papulo-pustular condition around the ankles extending up the leg for perhaps twelve inches. This was described to us by several patients as a sequel of dengue, but we are not sure whether this was not due to infected mosquito bites or to infection conveyed by scratching the irritable desquamating skin. *Jaundice* is said to occur in some cases and we have seen it in a few ourselves, but it has never been more than slight. It is of interest however in connection with the suggested relationship of dengue to yellow fever.

Diagnosis. From *influenza* the diagnosis rests chiefly upon the absence of coryzal symptoms, usually present with the so-called "influenza" seen in this country. As we have previously mentioned, the absence of coryza is a noticeable feature in dengue. Twenty-six

persons were specifically questioned on this point and all but one denied having any "cold in the head," "running at the nose," etc. Cough, again, which may be a feature of certain influenzal attacks, is usually absent. It was described in only seven out of twenty-four cases questioned. When present it is seldom more than a slight irritative cough, probably associated with the naso-pharyngeal congestion which is often present. The rash, and double phase temperature, and eye pains on movement, are important points when present.

From *scarlet fever and measles* dengue may be difficult to differentiate in isolated cases, and typical cases with a rash occurring early in an epidemic are often diagnosed as measles or scarlet fever. The coryza, nature and distribution of the rash, and Koplik's spots should generally, however, make a diagnosis of measles possible. The pulse in measles is rapid, in dengue often relatively slow. In scarlet fever the early vomiting, throat angina, type of rash, quick pulse and leucocytosis are important points.

The diagnosis from *yellow fever* is not of much importance in this country at present, but should be kept in mind in view of the possibility of the introduction of yellow fever into the *Stegomyia* infested part of Australia. *Jaundice*, though sometimes seen in dengue, is not frequent. Albuminaria is absent in dengue. The slow pulse of yellow fever which is used as a differential sign by Guiteras, cannot be employed with the dengue of Australia. The mild nature of the disease is a practical point when dengue is epidemic though we cannot exclude the possibility of mild cases of yellow fever appearing. In fact some observers have suggested that the dengue of Australia is really a modified yellow fever. The history of the disease and the known variability of dengue in various parts of the world, even in different parts of Australia, and the fixed mild character of the disease here, are arguments against this. It seems probable however that dengue fever is a disease closely related to yellow fever.

Acute rheumatic fever should be considered, but the localisation of pains in the joints and the absence of rash are usually sufficient. Inasmuch as other observers have described painful, hot swellings of the joints in some epidemics of dengue, it is possible that further investigation may disclose such cases in Australia. With one doubtful exception we have never seen any joint affections. This case was a child in the early febrile stage of some infection, who had pains and some swelling in several joints, but we were not able to follow the case further and do not know the final outcome. One of us diagnosed the case as "acute rheumatism."

III. MOSQUITOES AND DENGUE.

(*a*) *Previous work bearing on the experimental production of Dengue Fever by Mosquitoes.*

Graham (1903) reported experiments which are generally regarded as showing that *Culex fatigans* is able to convey the infection of dengue fever. In his experiments, four men slept under mosquito bars containing mosquitoes which had bitten dengue patients. Three cases of dengue resulted, four, five and six days after the first biting. The other case was unsuccessful. These experiments were conducted in a dengue district, and, to obviate the possibility of other means of infection in the infected district, Graham took mosquitoes to a mountain village where no cases of the disease had occurred and similarly infected there two persons, with incubation periods of four and five days respectively.

He admits that in many, perhaps in all, of his experiments *Stegomyia fasciata* were present amongst his mosquitoes. He seems to us, therefore, at most to have proved that *mosquitoes* can carry the disease, the variety or varieties remaining in doubt.

Graham gives also, but without convincing detail, the history of a case injected with the salivary gland of a *Culex*, which had fed on a dengue patient twenty-seven days previously. He says the patient "had a chill on third day and high fever, and an attack resembling in every way that of dengue, but so strong that I desisted from further experiments in that line." "That this was not septicaemia was proved by the finding of numerous dengue parasites in the blood." The second sentence suggests a doubt as to the diagnosis, and the finding of the "dengue parasites," which Graham discovered in his dengue cases, but which were probably artefacts, cannot be regarded as proof of the nature of the disease.

It is interesting to note Graham's remarks as to the distribution of *Culex fatigans* and *Stegomyia fasciata*. These appear to have been both plentifully present in Beyrouth, but on the higher parts *C. fatigans* was the principal mosquito, whilst in some villages there were few or no mosquitoes at all. As far as we can gather from his paper, the distribution of the dengue fever may have corresponded closer with the *Stegomyia* distribution than with the *Culex* distribution, but he has not analysed this point.

Bancroft's (1905) results were no doubt vitiated to some extent by the fact that he was working in an infected district. This may operate

in two ways. Firstly, his apparently successful cases might have acquired the disease in some other fashion, and, secondly, his failures might be due to the cases experimented with having passed through mild attacks of the disease previously. He had two apparently successful cases, the subjects of which were bitten by *S. fasciata* twelve and ten days after these had bitten dengue patients, whilst in the failures the persons bitten were bitten fifteen, fifteen and seventeen days after the mosquitoes had fed on individuals suffering from dengue. His experiments cannot be regarded as in any way conclusive, but are highly suggestive, and one is inclined to wonder that they have apparently not been repeated since. He notes that persons living in the country (non-infected districts?), visiting town friends with dengue in the daytime, acquired the disease, and deduces from this that if dengue is a mosquito-borne disease, *S. fasciata*, which is diurnal in biting habits, may be an efficient agent in the transmission.

Ashburn and Craig (1907) report one successful case in nine persons bitten by *Culex fatigans*. They regard three of these cases as not fair experiments, as proved later by unsuccessful intravenous injections of dengue blood, and another because he had possibly previously had the disease; the other three subsequently developed mild attacks of dengue on inoculation. One person was not bitten by the mosquitoes.

The mosquitoes used had been reared in captivity, and then fed on dengue cases. In the successful case the subject was exposed under nets on September 12th, 1906, to the bites of *C. fatigans*, which had bitten a patient with dengue on September 11th, 1906, but he was not bitten until the night of September 13th, and developed no symptoms until the night of September 17th. His temperature, however, rose on the 16th nearly twenty-four hours before. The incubation period would be from three and a half to somewhat over four days. The symptoms appeared to be fairly typical, and there was a slight rash on the abdomen and chest.

It is to be noted that the chart of this case shows the temperature to have been above the normal from the 13th September. This tendency to be above normal may be noticed in several of the charts of injected cases shown by these authors as occurring well before the onset of the fever.

The successful case was probably one of dengue, but arguing on analogy with yellow fever, the very short mosquito "ripening" period (less than two days) would make one accept it with reserve as originating from the mosquitoes. One cannot certainly exclude the possibility of

there being other sources of infection. Failing other evidence, the case is undoubtedly very suggestive of the possibility of *Culex* being a vector of dengue, but we can hardly understand the importance attributed to this isolated case by most text-books.

In reviewing the above series of experiments carried out by observers in three different parts of the world, it will be seen that as regards Graham's observations, whilst the evidence very strongly suggests that *Culex fatigans* is transmitting agent, this cannot be considered as being definitely proved on account of the probability that *Stegomyia fasciata* were included amongst the mosquitoes used.

The results of Ashburn and Craig are much more doubtful from the point of view of incriminating *C. fatigans*. Their mosquitoes apparently conveyed the infection so soon after having bitten a true case of dengue that no reasonable time could have elapsed to enable the organism of dengue to go through a phase of its life cycle in the mosquito. If their successful case arose from the bites of *C. fatigans*, and was not a case of natural infection, the most reasonable view to take is that in this instance the mosquitoes merely acted as infected lancets and not as true intermediate hosts.

Bancroft's experiments on the other hand very strongly support the view that *S. fasciata* transmits the disease, and are only vitiated by the fact that the experiments were conducted within the endemic and epidemic area.

REFERENCES.

ASHBURN and CRAIG (1907). Experimental Investigations Regarding the Etiology of Dengue Fever. *Phillip. Journ. of Sci.* II. 93.

BANCROFT (I. 1906). On the Aetiology of Dengue Fever. *Aust. Med. Gaz.* p. 17.

GRAHAM (1. VII. 1903). The Dengue: A Study of its Pathology and Mode of Propagation. *Journ. of Trop. Med.* p. 209.

A few further references not, however, of much importance will be found in Sticker, *op. cit.* [Editor.]

(*b*) *Australian mosquitoes as conveyers of disease.*

As both *Culex fatigans* and *Stegomyia fasciata* are common household pests in most parts of Australia which have suffered from this recent epidemic of dengue, it seemed quite probable that, if a mosquito were a vector of this disease, it might be one or other or both of these two species. *C. fatigans* is common in summer time in the southern districts of Australia where dengue does not occur, whilst the distribution of the disease is practically that of *S. fasciata*. Coupling these facts with the

observations and experiments of Dr Bancroft, greater suspicion naturally falls upon *S. fasciata* than upon *C. fatigans*. In our experiments both of these species were used. *S. fasciata* was found to bite freely in captivity in the day-time, but *C. fatigans*, though it did bite at night-time, was more shy and difficult to handle. In considering the transmission of the disease, a study of the habits of the mosquitoes in an infected area is important. Observations of the mosquitoes in general will show why it is that some species can readily transmit disease, whilst in the case of others disease transmission is unlikely. *C. fatigans* and *S. fasciata* are essentially domestic mosquitoes, thereby possessing increased facilities for transmitting diseases to human beings over "wild" mosquitoes. It is highly probable that both are introductions to Australia, having been non-existent here before the arrival of the white population. Both can apparently be easily conveyed from place to place by means of human agencies.

In this place it may be well to review shortly a few of the Australian mosquitoes which may play a part in conveying disease, or are present in exceptional numbers.

Culex fatigans Wied. This is the common domestic mosquito and is probably almost universally distributed throughout Australia. One of us (J. B. C.) has met with it abundantly in Sydney and in many country towns in New South Wales, and also in Adelaide. In the warmer parts of Australia it may probably be found biting throughout the year, but in the southern parts it disappears throughout the cold months, though during warmer evenings an occasional individual may be met with. It is essentially a night-biter and a feeder in the dark. We have never met with it biting during the day-time, but it occasionally bites in the evening in a poorly lighted room. Under these circumstances, it is more especially the legs or some other portion which is not exposed to the light that are bitten. The hum of the mosquito at night-time is very disturbing, the anticipation being more annoying than the bite itself, which in the cases of a number of individuals can hardly be noticed. Many of those bitten by the mosquito do not react by the raising of a wheal. At any time in bed the approach of the mosquito can usually be felt by the currents of air produced by the wings. This draws attention to the part where the mosquito settles, and, as it begins to feed, in many cases a slight but indefinite pricking sensation indicates the exact site. However quickly the hand is raised without disturbing the bedclothes, it is only rarely the movement is sufficiently quick to enable the mosquito to be destroyed. Its breeding place is in various

domestic water supplies—probably the cisterns of water closets may prove to be one of the most important of these. In places such as Sydney where during summer in some seasons there may be long periods without any rainfall, and in other seasons abundant rains for many days, the number of mosquitoes may be greatly increased under the latter conditions, indicating that breeding places form as a result of collections of rain-water. It has not yet been ascertained exactly where these outside breeding places are located in a city like Sydney, where, in the better residential localities, tins, broken bottles, and similar receptacles are not left lying about; but it is probable that places such as depressions in gutter-spoutings are some of the most important sites.

The distribution of this mosquito extends far beyond the areas in which dengue fever has occurred. For instance, though the mosquito is abundant in the neighbourhood of Sydney, no indigenous cases of dengue are known to have arisen in this city. Considering that imported cases of dengue have been not uncommon, the inference might be drawn that if *Culex fatigans* were capable of transmitting this disease, endemic cases should in consequence have arisen in Sydney.

Stegomyia fasciata Fabr. This species occurs in Queensland and extends into the northern coast towns of New South Wales. We have found it at Tweed Heads, Murwillumbah, Mullumbimby, Byron Bay, Casino and Grafton. Dr Ferguson has also identified specimens from Maclean and Tabulam.

Though the species has been recorded from Newcastle and from Victoria, there seems considerable doubt as to the identification, and in all probability specimens so designated were really *Scutomyia notoscripta*.

The insect is a day-biter, and during the recent dengue epidemic it was abundant in houses in the affected district, usually being more active in rooms that were dimly lighted. It was found breeding in water tanks, and in similar domestic supplies, one such source worthy of notice being open water in connection with acetylene gas installations. It is interesting to note that larvae were drawn off from the bottom of a tank which was four to five feet high, and which, as heavy rain had been falling for some days, was presumably full of water. In two or three jugs of water drawn off from the bottom, some larvae were obtained which afterwards hatched out.

We were able to confirm the statement that the eggs of *Stegomyia fasciata* can resist drying for some while and then develop under suitable conditions. In our second batch of these mosquitoes a number of eggs were laid in a small dish of water. On June 29th this dish had become

perfectly dry and was left exposed on a laboratory bench until August 30th, that is, during the end of winter and the beginning of spring. It was then immersed in water and in a few days some of the eggs hatched. Owing to the weather being cold the larvae developed very slowly, but early in November an adult which had recently emerged from its pupal stage was found floating on the surface of the water. At this period it was also noticed that a number of further eggs had hatched, the weather having become warmer. It would seem therefore that not only did the eggs resist two months absolute drying and then develop immediately on immersion in water, but that they also remained for some weeks without developing in this water until the weather became warmer.

A review of the above distribution of *Stegomyia fasciata* will show that the recent epidemic of dengue was nearly co-extensive with it. Thus, the epidemic appeared in all of the towns mentioned with the exception of Maclean and Tabulam, about which we have no information. It is interesting further to note that the epidemic extended southwards along the railway line, and this is doubtless explained by travellers becoming infected in one town and developing the disease in another, and there infecting the local mosquitoes and starting a fresh centre of the infection. Probably railway communication also facilitates the dispersal of *S. fasciata*. Though we did not find *S. fasciata* in railway carriages at Murwillumbah during the epidemic, we found them in the Station-master's office at Byron Bay. At Murwillumbah the mosquitoes found in the railway carriages were chiefly *Culex fatigans* and occasionally *Culicelsa annulirostris*.

Scutomyia notoscripta Skuse. This is a widely distributed mosquito throughout Australia, though we have never met with it in much abundance. It resembles very closely *Stegomyia fasciata* in its thoracic markings, but can be at once distinguished by a pure white band on the proboscis. It may occasionally be found biting inside houses. It is not known to be responsible for conveying any disease to human beings.

Culicelsa vigilax Skuse. This is the common bush mosquito so numerous at certain periods of the year in the neighbourhood of Sydney and other similarly situated districts. In places it is exceedingly numerous, as for instance in some of the creeks running into the Hawkesbury River where human beings may be attacked by hundreds of these insects at a time. The bite is rather painful and often raises small wheals. It is very interesting to compare the behaviour of this mosquito when attacking man with that of such domestic mosquitoes as *Culex fatigans* or *Stegomyia fasciata*. The two latter are exceedingly wary in their

habits, so that it is a matter of skill to kill them when they are attempting to bite. With *Culicelsa vigilax*, however, the insects settle on the hand or face and the finger can be slowly lowered down upon them and can crush them without disturbing them. *C. vigilax* is an Australian species accustomed to live in our bush, and probably to feed chiefly upon birds and marsupial hosts, which are unable to protect themselves against attacks of the mosquitoes by slapping them with hands.

Culicelsa annulirostris Skuse. Though a widely distributed mosquito in Australia and present in the area affected by the recent epidemic of dengue, there seems no reason at present to consider that it is responsible for the conveyance of any disease in man.

Nyssorhynchus annulipes Walker. This mosquito, the chief malarial transmitter in Australia, appears to be widely distributed throughout the continent, but in the southern parts as a rule only in small numbers. Here and there areas exist where it is present in sufficient numbers to be a source of danger should imported malarial cases reside there. In the coastal parts of Queensland, however, and in the Northern Territory, its incidence is sufficiently great to maintain in places endemic foci of malaria.

As regards the diseases spread or possibly spread by mosquitoes in Australia, malaria has already been mentioned. Our experiments on the conveyance of dengue have clearly proved that *Stegomyia fasciata* is responsible—is perhaps alone responsible—for the spread of this disease in Australia. The same mosquito, as is well known, is the transmitting agent of Yellow Fever. *Culex fatigans*, the common domestic mosquito, is a transmitting agent of *Filaria bancrofti*, and is responsible for the distribution of this disease in Queensland. So far there are no other diseases of human beings in Australia which are known to be transmitted by mosquitoes.

IV. REVIEW OF THE RESULTS OF THE MOSQUITO EXPERIMENTS.

A. *First Series of Mosquito Experiments.*

Details of our first series of mosquito experiments will be found in Appendix II. Four cases were bitten by *Stegomyia fasciata* and two by *Culex fatigans*. Negative results were obtained. There was, however, a very large mortality among the mosquitoes collected, and the bitings, except in one case, which received ten bites, were unsatisfactory.

B. *Second Series of Mosquito Experiments.*

In our second series of experiments mosquitoes were collected in Mullumbimby and the surrounding district, about 100 *S. fasciata* and 112 *C. fatigans* being thus obtained. The insects were collected from the hotel at which we stayed at Mullumbimby, from the post office, and from private houses in the town and district in which dengue fever cases had occurred—in some cases from the actual bedroom where patients were lying sick with the disease. A few mosquitoes were caught on the journey from Brisbane to Mullumbimby.

Occasionally *Culicelsa annulirostris* was found in Mullumbimby, and on our journey, but is not included in the above, and, with the exception noted in our *Culex fatigans* results, was not used in our experiments.

The *Stegomyia fasciata* and *Culex fatigans* were transferred to special cages, one containing *S. fasciata*, the other *C. fatigans*. The cages were made with a rounded opening, to which was attached a net sleeve. Through this the hand could be passed to add freshly caught mosquitoes and for the biting experiments.

At Mullumbimby, on May 8th, 1916, a dengue patient (*X*), who became ill on May 7th, was bitten by the *Stegomyia* then in the cage; on the 9th, he was again bitten by *Stegomyia*, and on the evening of the 8th, he was bitten by *Culex*. Both species of mosquitoes bit this patient well, and thus many of the Mullumbimby district mosquitoes had certainly been fed on the blood of a dengue case in the acute stage. Exactly how many mosquitoes bit this patient it is impossible to say, as this part of the work was conducted in a badly-lit bedroom.

On May 11th, 1916, these mosquitoes arrived in Sydney.

Biting experiments with the *Stegomyia* were conducted on May 11th, 12th, 13th, and 14th, and with *Culex* on May 11th, 12th, 13th, and 14th, as shewn more clearly later.

On May 15th, some seven *Culex* and eleven *Stegomyia* collected in the Grafton district, chiefly from houses of dengue patients, were added. Over 112 mosquitoes were collected in Grafton, of the following species: *Stegomyia fasciata*, twenty-seven; *Culex fatigans*, forty-six; *Culicelsa annulirostris*, thirty-seven; *Nyssorhynchus annulipes*, two. But although all care was taken, the mortality between Grafton and Sydney was large, and hence only this small number was added to the boxes.

Further feeding experiments were made with the remaining mixed Mullumbimby and Grafton mosquitoes, viz. with *S. fasciata* on May 15th,

16th, 17th, 18th, 19th, and 23rd; and with *C. fatigans* on May 15th and 16th.

On June 29th, the cages used in the experiments were emptied of the dead mosquitoes, and the remaining bodies that were not crushed were examined separately with a hand lens. Seventy-five *Stegomyia* were counted (two being males) in the *Stegomyia* cage; no other mosquitoes were found in this cage. Seventy-eight *C. fatigans* (two being males) and one *Culex annulirostris* were found in the *Culex* cage.

This procedure forms an additional check by another observer (J. B. C.) that the classification of mosquitoes was made accurately by B. B., and although about twenty-five *Stegomyia* were unaccounted for —probably they had been unrecognisably crushed in travelling, etc.— we can be reasonably certain that no *Culex* was included in the *Stegomyia* cage with which we obtained our positive results.

SUMMARY OF EXPERIMENTS.

The following is a short summary of the experiments made and results obtained with the mosquitoes, taking the nine persons volunteering *seriatim*:

Case I. *J. G.*, male, laboratory assistant (18 years), the subject of an unsuccessful *Stegomyia* biting experiment of the first series, was bitten on May 11th, 1916, at 2.15 p.m. by some twenty-eight *Stegomyia*. He remained well until the afternoon of the 19th, eight days later, when he noticed he had headache. That evening at 7 p.m. (eight days and five hours) he was again bitten by *Stegomyia*, and, while sitting with his hand in the cage, first became definitely ill. He passed through a typical attack of dengue fever, shewing a double temperature curve, rash, and symptoms described in detail below. Blood from this case reproduced the disease on injection. *Result positive.*

Case II. *McD.*, male, laboratory assistant, not previously the subject of experiment, was bitten on May 12th, 1916, by ten *Stegomyia* and on the 18th by three or four *Stegomyia*. He remained well until June 3rd, seventeen days from the second biting and twenty-two days from the first biting, when he had an influenzal attack with coryza for a few days, with no rash and nothing suggestive of dengue. *Result negative.*

Case III. *G.*, male, laboratory assistant, not previously the subject of experiment, bitten by about nine *Stegomyia* on May 13th, 1916, and by about three *Stegomyia* on the 17th. No symptoms have followed these bitings to date—July 14th, 1916. *Result negative.*

Case IV. *Wm.*, male, laboratory assistant, not previously the subject of experiment, was bitten by about thirty-six *Stegomyia* on May 14th, 1916 (mid-day), and by about thirty-six *Stegomyia* on the 15th (12.30 p.m. and 4.30 p.m.). On the 20th (six days and nine hours from first biting), whilst going to bed at night, he became ill and had a typical attack of dengue, with double temperature, rash and other symptoms detailed in Appendix III. His blood on injection reproduced the disease. *Result positive.*

Case V. *M.*, female, a nurse, was bitten by eighteen *Stegomyia* on May 16th, 1916 (noon) and became ill on the 25th, 10 p.m. (nine days ten hours later), and passed through a rather severe type of dengue with marked rash and double temperature. No blood was taken from this case for injection experiments. *Result positive.*

Case VI. *B. B.*, medical practitioner, was in dengue fever districts —Mullumbimby, Casino and Grafton—leaving Grafton for Sydney by boat on May 13th, 1916. To keep the mixed Grafton mosquitoes alive, he allowed them to bite him on the 12th and 14th, but remained perfectly well till the 23rd (2 p.m.); ten days after leaving the dengue district, he was bitten by fifteen *Stegomyia*. He remained well till May 29th, and his temperature was normal till May 31st, on rising at 9 a.m. (seven days and nineteen hours), he then became definitely ill and passed through a severe attack of dengue, with definite prodromal and secondary rashes, double temperature, and marked pains, etc., as described in Appendix III. Blood from this case reproduced the disease on injection. *Result positive*, but open to criticism since *B. B.* had been in a dengue district eighteen days before the attack developed.

Case VII. *W. T.*, bitten by one *Stegomyia* on May 12th, 1916. No illness followed. *Result negative.*

Case VIII. *M.*, a patient at a hospital, was bitten by *Culex fatigans* as follows: on May 11th, 1916, by about twelve; on May 12th and 13th by an unknown number; on May 14th, by at least twenty. *Result negative.*

Case IX. *J. O. S.*, laboratory assistant, a subject of *Culex* experiment in first series, was bitten by *C. fatigans* as follows: on May 15th, 1916, by two; on May 18th, by an unrecorded number. No symptoms followed. *Result negative.*

Discussion of Results.

In discussing the above results, it is important for the reader to bear in mind that our main object was to determine whether either or both of the mosquitoes experimented with were capable of transmitting

infection. We were quite in the dark, even if one or both species of mosquito were a transmitter of the disease, about a number of other important circumstances connected with such a possible means of transmission. The mosquito, if it carried infection at all, might or might not need a period to elapse after biting a patient before it became able to infect another person, and might remain infective for a period quite undetermined by us. Hence mosquitoes collected might not prove successful transmitters, not because they could not carry infection, but because they were not for one reason or another "ripe." Therefore, although our mosquitoes were collected in a district where dengue was prevalent, some from houses where patients were actually ill, and many from houses where patients had recently been ill, we felt it advisable to increase the chance of getting results by letting them bite patient *X.*, who had acquired the disease in the usual manner, on the dates mentioned. We were not in a position, and did not try, to solve the question of the "ripening" period, if any, nor of the period during which the mosquitoes remained infective.

Again, we deemed it advisable to have our first volunteers bitten more than once, and that because of the uncertainty as to whether the mosquitoes had "ripened," especially if infected from the known bitten patient, and because of the unknown time which the mosquitoes might remain infective. We foresaw that, to a certain extent, these multiple bitings might complicate our results and prevent us from obtaining the exact incubation period, but we attempted to arrange the experiments in such a way that we might hope to elucidate this point. As it turned out, the double biting has actually only interfered with the understanding of the incubation period in one case (*Wm.*), and the later volunteers, being only bitten once, tend to confirm, in this case, the longer incubation period rather than the five days five hours period which may have been the incubation period for *Wm.*

That we have succeeded in proving the principal hypothesis, the possibility of transmission by mosquitoes, depends mainly on the satisfactory nature of the evidence that our apparently successful cases were really instances of dengue fever. If we are successful in this there seems no escape from the conclusion that transmission had occurred through the agency of the *Stegomyia fasciata* used by us.

We have not, in our opinion, shewn conclusively that *Culex fatigans* may not also spread the disease, though we think this unlikely.

That the disease which followed the biting of our volunteers by *Stegomyia* was dengue there can be no reasonable doubt when the

following circumstances are considered. In the four successful cases the illness began at a period of from six (possibly five) to nine days after being bitten, an incubation period the limits of which were the same as those of cases of dengue fever conveyed by blood inoculation from previous cases. The symptoms, signs and clinical characteristics of the disease in the four successful mosquito cases were indistinguishable from those in attacks of dengue naturally contracted. The rashes in two of the cases were typical of those seen in certain dengue cases, and could not be confounded with those of measles or scarlet fever, the other febrile complaints with which a distinct rash is usually associated. After the rashes disappeared, these two patients suffered from intense itching of the parts which had been affected by the rash, in one case to such an extent as to be almost unbearable. Such intense pruritus, rendering life temporarily a burden, has occurred in some instances in the North Coast district of this State following the disappearance of the rash of dengue. This itching, following a febrile complaint accompanied by a rash, we consider to be almost pathognomonic, when it occurs, of dengue.

Of other noteworthy features characteristic in general of attacks of dengue fever, the following may be noted. All the four patients shewed a swollen, hot-looking condition of the face, with a flushed, red suffusion, resembling somewhat that seen in the incipient stages of measles, or after an alcoholic bout. They all had, in fact, what may be called the "dengue face." All the cases shewed a sudden onset, more or less characteristic of dengue, and not so common in other febriculae. In all there was a distinct tendency to a double rise of temperature, the early rise being followed by a fall for a few days and then by a final rise. The blood examinations made during the course of the disease shewed a definite leucopenia, a characteristic feature of dengue.

In the three cases in which blood was taken during the height of the disease and injected into volunteers who had never been in contact with the patients, the disease was successfully transmitted after the usual incubation period.

From the above summary it is clear that the disease in the four volunteers was not measles, German measles, scarlet fever, or any of the other acute infective fevers accompanied occasionally by similar rashes, such as the early stage of small-pox.

In all large communities there are continually present febrile complaints not accompanied by definite rashes, which are loosely styled "influenza." These vary much from time to time, and probably represent a number of distinct entities, with features so little characteristic,

and symptoms so mild and evanescent, that it has not been possible as yet to differentiate one from another. Many of these are accompanied by a definite coryza, which was absent in our experimental cases. During the course of our experiments, such complaints were not absent from Sydney, and though in specific instances individual cases might resemble aberrant cases of natural dengue, none could be considered as typical cases, such as were our volunteers, and rashes did not develop.

Having established that the disease occurring in our four volunteers was dengue fever, it is necessary to shew beyond reasonable doubt that the disease developed as a result of the bites of certain infected mosquitoes, *Stegomyia fasciata*. As two of the four individuals had never been in a dengue area, while a third had been away from such an area for eight years, and as the experimental bitings took place in a district in which dengue is unknown, except as imported cases, and as we know of no other means by which they could have become infected, no other conclusion is left save that the *Stegomyia* transmitted the disease. It is true that one of us, who contracted the disease naturally, had been in more or less daily association with two of these three volunteers, and had seen the third for a few minutes, but it is hardly reasonable to suppose that he should have carried infection to these three persons and to these three only, and yet have failed to convey infection to other members of the staff and to his own household. The fourth volunteer, one of us (*B. B.*), had returned recently from a dengue area. It might, therefore, be suggested that the disease from which he suffered was naturally contracted there. It will be noted, however, that he had been away from the dengue area for a period of time far exceeding the established limits of the incubation period, so that, were his case one of natural infection, then the incubation period, in his case, of a typical attack must be considered to be twice as long as our results in other cases would indicate.

As further shewing that the disease developed by the four volunteers is to be attributed to the bitings of the mosquitoes, is the fact that, though each volunteer was bitten on different days and with varying intervals between them, the incubation periods of their complaints fell within the time found to be the incubation period in our blood inoculation experiments. Such results in four instances must be considered more than mere coincidences.

That we were not successful in conveying the disease to all the volunteers is not to be wondered at. These other persons were certainly not so extensively bitten as were the successful cases. As

perhaps only a certain number of mosquitoes were infective, and as mosquitoes engorged with blood one day, whether infective or not, may not feed again perhaps for several days, it can be understood how such failures can occur, whilst the opinion that there may be a possible minimum amount of infective material necessary to ensure successful inoculation by bites of the mosquitoes is another hypothetical explanation.

Apart from this, the positive results obtained in the four successful cases overshadow entirely the three negative results, which need only be considered from the theoretical point of view as to why the patients did not develop the disease, and not from the practical point of view, as to whether or not *Stegomyia* is the vector.

Incubation Period. We are able, fortunately, to draw reasonably accurate conclusions, even from the first doubly bitten cases, as to the incubation period.

Case I. *J. G.* became ill while actually being bitten for the second time. His is obviously an eight days' incubation period. *Case IV.* *Wm.*, the second successful case, was bitten on two successive days, and his incubation period would be six days and nine hours, or five days five hours, depending on whether we count from his first or second biting. In the case of *Nurse M.* the incubation period is definitely nine days ten hours, and in the case of *B. B.* about seven and three-quarter days, if we count from the first rise of temperature, and about five and three-quarter days if we count from the first feeling of malaise.

This gives us for our mosquito cases an incubation period of approximately six to nine and a half days, possibly five and a quarter to nine and a half days.

General Conclusions from Series II.

(1) *Stegomyia fasciata* caught in a dengue infected district in the surroundings of cases of the disease, some of them known to have fed on a dengue patient on the first and second days of his illness, transported to a non-dengue district, reproduced the disease in four out of seven persons on whom biting experiments were conducted.

(2) Blood taken from three of these four cases reproduced the disease when injected into further persons. The blood of one case was not tested.

(3) The incubation period of the four cases was found to be possibly between five and nine and a half days, probably between six and a half and nine and a half days, counting from the biting to the definite onset.

(4) No known case of contagion occurred from any of the above four cases.

(5) No evidence was obtained from two cases, one of which was heavily and repeatedly bitten with *Culex fatigans*, that this mosquito can transmit dengue fever.

V. REVIEW OF THE RESULTS OF THE INOCULATION AND ALLIED EXPERIMENTS.

(*a*) *Clinical description of cases artificially inoculated in Sydney.*

These observations on the clinical phenomena of Dengue Fever are based on the results of thirty-two experimental inoculations, etc., for the transmission of the disease, made at the Rookwood State Hospital and Asylum. Thirty patients who volunteered for the experiments were treated in various ways.

Of the twenty-eight *inoculations* thirteen experiments gave positive results, the patients developing what we regard as undoubted dengue fever; twelve experiments gave definitely negative results; and seven gave doubtful results. We discuss the doubtful and negative cases elsewhere. In many of these the nature of the experiment led one to expect a negative result. The clinical description of the thirteen successful cases may be discussed in detail.

The incubation period is reckoned as the time elapsing between the date of inoculation and the appearance of the initial symptoms or sign. Omitting four positive cases which had more than one injection and in which the incubation period is not quite definitely established, the other nine positive cases gave the following results: five to six days—three cases; six to seven days—two cases; seven to eight days—one case; eight to nine days—three cases. Hence the incubation period ranged from five to nine days. Five gave periods between five and seven days.

The onset was usually sudden, the symptoms, at first mild, becoming well-defined within a few hours. The most consistent initial symptom was *headache*, usually occipital, less often frontal, rarely general. In only one case was headache absent. A few had dizziness and most complained of a "shivery feeling" in the early stages.

The temperature rose fairly rapidly from the beginning. On one occasion the temperature, and not the headache was the earliest sign of infection; whilst in four cases the temperature and headache were

practically coincident in time of appearance. Usually the temperature rose so rapidly that the maximum was attained on the first or second day. The temperature curve shewed some degree of fluctuation with a rapid subsidence, the fall being practically by crisis. Two of the cases shewed the typical diphasic temperature charts. Four shewed irregular diphasic charts; four shewed irregular charts; and two shewed definitely monophasic charts. One case relapsed and shewed a monophasic variation in the first attack and a diphasic variation in the relapse. The highest temperature recorded was 104° F. (average 102°–103°).

The pulse rates in the inoculated cases form a very interesting study, but we wish to make clear from the start that our records are open to criticism from several points. Most of our cases were men close to or over 50 years of age, and all were inmates of an Asylum and may thus be regarded as of a selected type, and these may normally have pulse rates different from the normal active man of the outside world. Again, we have only the pulse record *after* the onset of the illness, and have not been able to control such pulse rates with the rates before the injection or other exhibition of presumably infectious material. We therefore present our own results with due reserve. We are inclined to think from some of the records of doubtful or negative cases that some degree of absolute bradycardia may be a feature of the pulse in such type of persons. We have submitted thirteen cases which we regard as having suffered from experimental dengue to detailed analysis, and have analysed the pulse-temperature ratio—(1) in the first stage of the fever; (2) in the second stage of the fever (in cases where the diphasic temperature variation was not clear we have made observations in early and later stages of the febrile phase); and (3) in the post-febrile condition. In Case 25 which we regard as an instance of relapse, the periods analysed were in the first monophasic febrile phase, in the inter-febrile interval, in the first and second stage of the relapse, and in the post-febrile phase. Summarising these results as well as possible in these thirteen cases we may say that:

1. Eleven out of the thirteen shewed during the febrile stage or stages marked *relative bradycardia*. One case shewed periods of *definite absolute bradycardia*.

2. In what may be roughly regarded as the first febrile paroxysm, five cases shewed marked *relative bradycardia*, and seven shewed slight *relative bradycardia* (in one case there was no record).

3. In the second febrile stage or in the latter part of an irregular febrile attack, nine cases shewed marked *relative bradycardia*; two shewed

slight *relative bradycardia*, and in two definitely monophasic charts there is of course no record.

4. In the post febrile phase there was marked *absolute bradycardia* in one case (pulse going as low as 42); *definite absolute bradycardia* (pulses at times below 50–55) in six cases; *slight absolute bradycardia* (below 50–60) in four cases; normal pulse in one case and no record in one case.

With the reservations above mentioned it appears from our results (refer to table overleaf) that:

1. There is a tendency to slow pulse in our infected cases of dengue which may manifest itself by absolute or relative bradycardia.

2. Relative bradycardia is a remarkable feature of the latter part of the febrile paroxysm, or of the second phase when it occurs. It may occur from the onset of the fever but is usually in the early stages less marked.

3. Absolute bradycardia is found in many cases in the post-febrile stage. It is generally intermittent in type and alternates with normal or quick pulse rates. It may be also found during the febrile stage.

The appearance of the patient was at once suggestive. The face was flushed, with a suggestion of puffiness about the features, and the conjunctivae were injected. The injection of the eyes and of the pharynx was constant. The facies is comparable to that of a man in the early excitement of alcoholic indulgence, and is not unlike the facies of measles. The patients looked bright and alert, and made little or no complaint even in the cases shewing marked reactions and notwithstanding that no analgesic or antipyretic drugs were used. Although the type of patients volunteering for this work would perhaps be less likely to complain than those of a finer mould, this comparative indifference to the disease is worthy of record, seeing that in the Murwillumbah epidemic the observers describe the facial expression as one of suffering and state that it was necessary in a few cases to resort to morphine. We should, however, correlate the mildness of the symptoms in our experimental cases with the fact that as soon as the patient was found to be febrile he was put to bed and kept there till the attack was over, whereas in many of the severe natural cases seen by us the patients had for a while attempted to carry on their work.

Pain in these cases was not an important feature. The pains complained of were attributed in most cases to "aching muscles" and the common sites were the lumbar region, nape of the neck and limbs. Two subjects complained of "rheumatic pains" but the joints were neither

The Pulse in Experimental (Injected) Dengue.

Review of the Pulse Variation in the Inoculated Cases.

Case	Age	Type	Pulse or febrile phase: 1st	Pulse or febrile phase: 2nd	Post-febrile phase
4	46	Irregular	Irregular marked relative and definite absolute		Definite absolute irregular
6	48	Diphasic	Sl.R.	M.R.	Normal
7	49	Diphasic*	Sl.R.	M.R.	Definite
9	56	Diphasic	(No record) ...	M.R.	No record
11	—	Monophasic	M.R.	— ...	Slight
12	47	Diphasic*	M.R.	M.R.	Marked A
13	44	Irregular	Sl.R.	M.R.	Definite
16	55	Diphasic*	Sl.R.	Sl.R.	Slight
17	38	Irregular	Irregular slight relative		... Definite
25	52	Relapsing			
		1. Monophasic ...	Sl.R.	Sl.R. M.R.	Slight
		2. Diphasic	Sl.R.		
26	67	Monophasic	M.R.	—	Definite
27	50	Irregular	M.R.	M.R.	Definite
29	46	Diphasic*	Sl.	M.R.	Slight

Summary:		Relative bradycardia				Absolute bradycardia	
Typical Diphasic	2	Slight	7	Marked	9	Normal	1
Irregular Diphasic	4	Marked	5	Slight	2	Slight	4
Relapsing	1	No record	1	No record (Monophasic)	2	Definite	6
		Absolute bradycardia					
Monophasic	2					Marked	1
Irregular	4	Irregular	1			No record	1

S.R. = Slight relative bradycardia. M.R. = Marked relative bradycardia.
M.A. = Marked absolute bradycardia. * = Irregular.

swollen nor tender to touch. Two patients suffered no pain at all. In one or two cases the pains dominated the picture, but even then they were not severe, making the patient unhappy merely for a day or two.

The appetite was usually good and unimpaired. Only one patient complained of anorexia.

Five patients suffered from *nocturnal sweating*. The sweating was generally profuse. These sweats usually recurred for a few nights. One of us (*W. McD.*) records that they bore no relation to the temperature, coming on quite independently of its rise or fall, but examination of some of the charts suggests a relation between sweating and abrupt temperature falls.

Most of the patients slept well, but in four cases *sleeplessness* was complained of on occasional nights.

The rash. Some degree of rash was an almost constant feature, being absent in only one of the positive cases and doubtful in two cases.

The prodromal rash was sometimes difficult to detect. In seven cases some more or less definite rash or eruption was noted. *The later eruption* was found in ten cases and absent in three cases. We have recorded the first appearance on days from the second to the seventh day of the disease, usually on the third or fourth day. The rash lasted a variable time being often visible ten days or more after its appearance. In many cases it is difficult to distinguish between the preliminary and later rashes, and the distinction does not seem a very useful one. Out of the thirteen cases considered the later rash was very distinct in four cases, definite in four cases, slight in two cases, and negative in three cases.

Character of later rash. In some cases the rash was polymorphous. In two cases it was morbilliform. Most commonly it was a pinkish erythematous mottling with irregularly shaped areas (sometimes definitely raised), of varying intensity of colour, surrounding islets of white. In one or two cases the rash covered the whole body, being apparent even on the soles of the feet; but in the main the distribution favoured the upper portion of the body, viz., the back, chest, abdomen and upper arms. Itchiness was sometimes complained of. A constant sign was a congested erythema of the back. This was present in cases shewing no rash. In a few cases the rash persisted and was still visible when the patients were discharged.

Enlarged lymph-glands were palpable in two patients.

Vomiting in the early stages of the disease was observed in two patients. Four patients complained of a cough without expectoration.

The urine analysis gave fairly constant results. The specific gravity varied from 1010 to 1025; the reaction was acid and there was no albumen. In two cases the specific gravity rose suddenly during two days to 1030 and a reduction of Fehling's Solution took place. Following on this, the specific gravity of the two urines fell to 1006 and 1017 respectively, and no glycosuria was detected. Albumen was absent in every case. In this respect, the disease may be contrasted with Yellow Fever, in which albuminuria is so distinctive a feature.

The duration of the disease ranged from four to seven days, though most of the cases were kept in bed for ten days, and the rash persisted at times for two to three weeks. Convalescence was rather protracted and a number of the patients complained of weakness persisting for some time.

The diagnosis would be readily made in an epidemic but would admit of some difficulty in sporadic cases. The sudden onset with headache; the flushing of the face and injection of the eyes and pharynx; the congested erythematous appearance of the back; the occurrence of the rash; and the condition of the pulse—all unite to form a more or less typical picture. Perhaps it would be necessary to differentiate the condition from Influenza, Fibrositis, and Measles.

In all the cases recorded above, the toxaemia would appear to have been slight, as, although the symptoms were well-marked, there was never any anxiety as to the ultimate complete recovery of the patient.

No case of infection occurred other than amongst the artificially inoculated.

Incidence of Symptoms and Signs in the Thirteen Positive Cases.

Headache	11
Vomiting	2
Cough	4
Sleeplessness	4
Aching eyes	5
Rash { Preliminary	5
Rash { Terminal	10
Flushing of face...	12
Relative Bradycardia	13
Absolute Bradycardia	11
Typical Diphasic temperature chart ...	2
Irregular Diphasic temperature chart ...	4
Monophasic temperature chart ...	2
Irregular temperature chart	4
Relapsing temperature chart	1
Sweating	5
Pains { Aching muscle pains	8
Pains { Joint pains	2
Anorexia	1

Marked weakness during convalescence—Several cases.

(*b*) *Consideration of Cases* 1 *to* 9.

These cases, with the exception of Case 1, all received two injections of material separated by an interval of four days. Case 1 received only the first injection, but it is considered with these other cases because the material was the same as that used for the first injection in some of the other cases.

At the beginning of our work in connection with the experimental transmission of dengue from one case to another our efforts were at

Table shewing incidence, date of appearance, etc., of rash.

Case no.	Date of onset	First examination			Later rash				Remarks
		Date	Day	Rash	Date	Day	Duration	Intensity	
4	19 . iv . 16	20 . iv . 16	2	+ ?	+	–	+	Neg.	Doubtful early erythema. No later rash.
6	16 . iv . 16	17 . iv . 16	2	Neg.	18–19 . iv . 16	3, 4	11	+S	Prodromal rash not noted. Slight later rash.
7	16 . iv . 16	17 . iv . 16	2	+S	18 . iv . 16	3	6	+	Slight prodromal rash. Definite later rash.
9	19 . iv . 16	20 . iv . 16	2	+ ?	25 . iv . 16	7	7	+ +	Doubtful prodromal rash. Marked later rash.
11	23 . iv . 16	23 . iv . 16	1	Neg.	24 . iv . 16	2	11	+ +	No prodromal rash. Marked later rash.
12	25 . iv . 16	25 . iv . 16	1	+	27 . iv . 16	3	17	+ +	Definite prodromal and marked later rash.
13	25 . iv . 16	25 . iv . 16	1	+S	27 . iv . 16	3	11	+	Slight prodromal and definite later rash.
16	3 . v . 16	3 . v . 16	1	+ ?	+	–	+	Neg.	Doubtful early erythema. No later rash.
17	3 . v . 16	3 . v . 16	1	Neg.	7 . v . 16	5	5	+	No prodromal rash. Definite later rash.
25	21 . v . 16	22 . v . 16	2	Neg.	23–26 . v . 16	3, 6	11	+	No prodromal rash. Definite later rash.
26 (32)	11 . vi . 16	11 . vi . 16	1	Neg.	13 . vi . 16	3	13	+S	No prodromal rash. Slight later rash.
27	1 . vi . 16	2 . vi . 16	2	Neg.	+	–	+	Neg.	No rash noted.
29	30 . v . 16	31 . v . 16	2	+ ?	1 . vi . 16	3	15	+ +	Doubtful early erythema. Marked later rash.

Day = Day of Disease. + + = Marked. + = Definite. +S = Slight. Neg. = Negative. + ? = Doubtful.

first directed to establishing in Sydney transmitted cases of the disease by some means or other. Nine volunteers were secured who for a consideration submitted themselves on April 8th, 1916, to injections of material from the bloods of two natural cases of the disease, which had occurred at Murwillumbah. Two or three days later the occurrence of a natural case of the disease at the Coast Hospital, in which the infection had been contracted in the North Coast district, gave us a further opportunity of obtaining infective material, though in this case the patient was convalescing, being in the eighth day of the disease. Material from this second source was injected on April 12th, 1916, into eight of the nine volunteers who had received the first injection four days previously. As the results shewed it is to be regretted that in any case one individual received two separate injections within such a short interval of time. The difficulty of obtaining volunteers together with the desire on our part at this stage of our work to obtain by any means that could be compassed a strain or strains of the disease in Sydney under our control together with the belief that at that time the incubation period of about four days as given in the text-books was the correct one and that therefore our first injections had failed to produce any result, all contributed to our using so many of the same volunteers for the second injection. Coupled with this was inadvertence on the part of the two of us responsible for the planning out of these experiments in not making it clear that it was advisable to make every possible endeavour to obtain new volunteers to supplement as far as possible previous ones.

The two injections, however, having thus been made with an interval of four days between them, it is necessary to consider what information can be reasonably gathered from the results obtained, either taken alone or taken in conjunction with the other experiments carried out by us. Considering the first injections made on April 8th, Cases 1 to 5 received subcutaneously 1 c.c. Pasteur-Chamberland filtrate from the citrated blood of the Natural Case A taken on the third day of the disease at Murwillumbah. The citrated blood had been outside the body for three days previous to injection. Of these five cases Case 1 received first injection only, and developed no signs of illness whatsoever. Cases 2, 3, 4 and 5 received on April 12th 1 c.c. of citrated blood from the Natural Case C, taken on the eighth day of the disease. Cases 2 and 4 shewed no symptoms of the disease at any time. Case 3 developed an illness of a doubtful nature beginning twenty-two days after the first injection and eighteen days after the second injection. Case 4 developed an

apparently definite attack of dengue of a mild type, eleven days after the first injection and seven days after the second injection.

Cases 6 and 7 received as their first injection serum and corpuscles taken on the third day of the disease of Natural Case B occurring at Murwillumbah, the material having been outside the body for four days. Four days later Case 6 received a subcutaneous injection of serum taken on the eighth day of the natural disease from Case C, whilst Case 7 received citrated blood from the same Case C. Cases 6 and 7 developed typical attacks of dengue twelve days after the first injection, and eight days after the second injection.

Cases 8 and 9 received on April 8th subcutaneous injections of clear serum obtained from blood taken on the third day of the natural disease from Case B at Murwillumbah, which material had been outside the body for four days. In addition Case 8 received on April 12th citrated blood taken on the eighth day of the natural disease of Case C, whilst Case 9 received an injection of serum from this same Case C. Case 8 remained well throughout, whilst Case 9 developed a typical attack of dengue eleven days after the first injection, and seven days after the second injection.

A summary of the above results shows that of the five cases injected with the Pasteur-Chamberland filtrate of the citrated blood of natural Case A, three remained well throughout, Case 3 had an illness of a doubtful nature beginning twenty-two and eighteen days respectively after the injections, whilst Case 4 developed dengue eleven and seven days respectively after the injections.

Cases 6 and 7 injected primarily with the serum and corpuscles of Case B both developed typical attacks of dengue eight days afterwards, and four days after the second injection. Of Cases 8 and 9 injected in the first case with the clear serum of Case B, Case 8 remained well throughout and Case 9 developed dengue eleven days afterwards, and seven days after the second injection.

Of Cases 2, 3, 4, 5, 7 and 8 receiving as their second injections citrated blood from Case C taken on the eighth day of the disease, Cases 2, 5 and 8 developed no disease, Case 3 developed a doubtful disease eighteen days after the second injection and twenty-two days after the first, Case 4 developed dengue seven days after the second injection and eleven days after the first, whilst Case 7 developed dengue eight days after the first injection and four days after the second. Of Cases 6 and 9 injected secondarily with the serum of natural Case C taken on the eighth day of the disease, Case 6 developed dengue eight

days after the first injection and four days after this the second injection, whilst Case 9 developed dengue seven days after this second injection and eleven days after the first injection.

It is to be noted that in the only two cases, viz., Cases 6 and 7, receiving injections of the *serum and corpuscles from Case B*, both developed dengue eight days later whilst their second injections consisted in the first case of serum from Case C, and in the second case of citrated blood from Case C. As the only other case (Case 9) which received serum from Case C as a second injection did not develop dengue until the seventh day after this injection, and as of the five other cases which received citrated blood from Case C, as second injections three remained quite well, one (Case 4) developed dengue seven days after this injection, and the remaining one developed an indefinite disease later, it is reasonable to infer that Cases 6 and 7 were infected with the material used in the first injection giving an incubation period of seven days. If this view be correct it cannot be stated whether the second injections also contained infective material, as this would be masked by the positive results from the first injections. The other two cases in this series in which typical dengue developed had one common factor, viz., that they both received injections, in one case (Case 4) of the citrated blood and in the other (Case 9) of serum, from Natural Case C as second injections. The disease appeared in each seven days later. That in this case it is reasonable to attribute the disease to the second injection and not to the first is shewn by the fact that, as regards Case 4, of the four other cases receiving a similar *first* injection three remained perfectly well, whilst the fourth developed an indefinite disease many days later; and that, as regards Case 9, the only other case receiving a similar first injection was Case 8, which remained well throughout. The only other case which received as a second injection serum from Case C was Case 6 in which the infectivity of this material may have been masked by the presumed infectivity of the material first injected.

As our further experimental results shew, in no instance have we found an incubation period as low as four days, or as high as eleven days. On these results the development of dengue in Cases 6 and 7 must be attributed to the first injection, giving an incubation period of eight days, whilst the disease in Cases 4 and 9 must be attributed to the second injection, giving incubation periods of seven days.

(*c*) *Cases shewing that the virus exists in the blood* (*serum* (*and*) *or corpuscles*).

Owing to the difficulty in preventing blood from clotting, and the necessity of doing a Wassermann reaction before injecting the blood from one person into another, no attempts were made by us to convey blood directly from one individual to another in its natural state. In certain experiments, the serum and corpuscles of blood which had been taken and allowed to clot, were injected subcutaneously, whilst in other cases whole blood was received into citrate normal saline solution, and this, or certain portions of it, were injected.

It is unnecessary to labour the point that the virus exists in some constituent of the blood. The interesting point to ascertain is whether the virus exists in the serum or is in some way attached to the corpuscles. The following cases show that the virus is present in a mixture of serum and corpuscles from clotted infective blood, namely, Case 13, Case 26, Case 27, and Case 29. In Case 28 the result was doubtful, the only indication of a possible mild attack of dengue being a slight rise of temperature for a few days beginning on the fifteenth day. As noted in a special discussion on Cases 2–9 the positive results in Cases 6 and 7 we are inclined to attribute to the injection of mixed serum and corpuscles.

(*d*) *A case inoculated with whole citrated blood.*

If the positive result in *Case* 4 is to be attributed to the second injection consisting of citrated blood from a natural case of the disease taken on the eighth day of that disease (*vide* discussion on *Cases* 2 *to* 9), as is suggested by the length of the incubation period, then citrated blood as a whole, as might have been expected, is infective, and treatment with citrated normal saline solution is not injurious or at least lethal to the virus.

(*e*) *Cases in which the Serum of clotted blood was used for injections.*

Of four cases receiving this material, three gave positive results, and one a negative result.

In *Case* 11 in which a positive result followed, the blood was taken on the morning of the third day of the disease and was injected into the volunteer on the evening of the same day. A typical attack of dengue with its rash developed.

In *Case* 25 in which a positive result also followed, the blood had been taken on the second day of the disease and had been kept about eight days in an ice chest before injection. A typical attack of dengue fever resulted.

Case 9, which also developed a typical attack of dengue, received injections of serum, with an interval of four days between them, from two separate sources. *Case* 8 in which a negative result was obtained, received the same first injection of serum as *Case* 9, and four days later a second injection consisting of citrated blood from a different case of the disease. No ill effects followed. From these results in *Case* 8 we attribute the positive result in *Case* 9 to the second injection of serum, the second injection in these cases being the factor in which they differed.

(*f*) *Cases shewing the experimental results with Washed Corpuscles.*

Three cases each received a subcutaneous injection of washed corpuscles from cases of dengue. The corpuscles had been obtained by withdrawing blood from a vein and injecting it immediately into a solution of citrate of soda in normal saline solution. Thereafter the mixture was centrifuged, the supernatant fluid pipetted off and the deposit of corpuscles shaken up with fresh normal saline solution and re-centrifuged. This was repeated from four to seven times. With such material two cases gave negative results, and one a not quite conclusive positive result. In *Case* 10, giving a negative result, the blood had been taken on the third day of the disease, and the corpuscles were injected into the volunteer within twelve hours of removal. Serum from this case derived from the same sample of blood gave rise to a typical attack of dengue fever (Case 11) showing that the blood at this period was infective.

In *Case* 14, which was also negative, the blood was taken on the fourth day and injected into the volunteer the succeeding day. The citrate washings from this case, as detailed later, also gave a negative result in Case 15.

In *Case* 16 an apparently positive result followed. This blood was taken on the second day of the disease and injected into the volunteer on the following day. This illness was a mild one beginning about five days and twenty hours after injection. The patient's appearance and symptoms were those of a mild attack of dengue fever; the temperature reaction was mild, and there was no definite rash. In our opinion the case was a mild one of dengue, though it must be considered as open to considerable criticism.

As inoculation of other material shows that blood still remains infective on the third (and fourth?) days of the disease, the failure of the washed corpuscles to produce the disease in Cases 10 (and 14) and an apparently successful result in Case 16, cannot be considered as dependent on Cases 10 and 14 receiving blood from patients in the third and fourth(?) days of the disease respectively, whilst in Case 16 the patient was only in the second day of the disease. These anomalous results seem rather to indicate that the virus is not of necessity intimately associated with the corpuscles, though it may temporarily adhere to them and be sometimes successfully removed by thorough washing. In other words, these results tend to support the view that the parasite, whatever it is, is not intracorpuscular. If Case 16 be rejected as not being a mild case of the disease, this view is still more strongly supported. On the other hand, if Case 16 be considered a mild case of the disease, the mildness compared with the very definite attack in Case 17, which received the citrated plasma from the same blood, may be considered as shewing that the virus may become attached loosely to the surface of the corpuscles from which by washing it may be to some degree detached, though sufficient virus may still adhere to produce a mild attack of the disease.

(*g*) *Cases in which the fluid part of citrated blood was injected.*

In these cases the blood was received into citrate normal saline solution and then centrifuged. The supernatant fluid was then pipetted off and used for injections. As the centrifuge used was not of very high speed, it cannot be considered certain that the fluid injected was free from corpuscles though these must have been reduced to a minimum. Of two cases receiving these injections, one gave a typical positive result, and one a negative one.

Case 17 (a positive result) followed the use of material obtained on the second day of the disease. Case 16, which received the washed corpuscles of the same blood, developed apparently a mild attack of dengue. Thus the washings of the corpuscles from Case 13 gave a very definite attack of dengue fever in Case 17, as compared with the mild, somewhat doubtful, attack resulting from the washed corpuscles in Case 16. Case 15 gave a negative result. The material in this case was received from Case 11 on the fourth day of the disease. The washed corpuscles from the same blood also gave a negative result in Case 14.

(*h*) *Cases in which a Pasteur-Chamberland Filtrate of the serum and corpuscles obtained from clotted blood was injected.*

Of the five cases in which such a filtrate was inoculated subcutaneously, four gave a negative result and one a positive one.

Case 12 which gave a positive result was injected with the filtrate of the clot and serum obtained from blood taken on the second day of an attack of dengue. It was injected the day after collection and was followed by a typical attack of dengue. Unfortunately in this case a test was not made of the reliability of the candle used by inoculating the serum beforehand with a suspension of *B. prodigiosus*. The untreated serum and clot likewise gave a positive result. *Case* 18 which was negative, received the filtrate of the clot and serum from blood taken on the fifth day of a severe case of dengue, the blood having been kept on ice for four days before filtration and the material used on the sixth day from the time of collection. *Case* 19 which was negative received a filtrate of the serum and clot from blood from a case in the fourth day of the disease, the blood being taken one day and the filtrate injected two days later. A local reaction of the arm followed the inoculation, but no attack of dengue. *Case* 20 received a filtrate of the serum and clot from blood taken within forty-eight hours of the onset of an attack of dengue and injected eight days later, having been kept on ice meanwhile. *Case* 21 which was negative, received an injection of the filtrate of the serum and clot from blood taken within forty-eight hours of the onset of an attack of dengue, but which was kept in an ice chest for eight days before injection. In *Cases* 18, 19, 20 and 21 the efficacy of the Pasteur-Chamberland filter was tested by its withholding *B. prodigiosus* added to the serum and clot before filtration.

As regards these negative cases, *Case* 19 received the filtrate from *Case* 11. *Cases* 14 and 15 received respectively washed corpuscles and the citrated washings of these corpuscles from the same case and from the same sample of blood; both of these cases also gave a negative result. This blood was taken on the fourth day of the disease in *Case* 11. These three negative results would seem to indicate that the blood of this case on the date in question was non-infective.

In *Case* 18 the blood was taken on the fifth day of the disease, and there were no other cases inoculated with other samples of this blood to shew whether it was still infective.

In *Case* 20 the blood was taken at a very early period of the disease, —a period during which we know that it is infective. It was kept

outside the body at a low temperature for eight days. In this case the length of time for which the material was kept outside the human body may have tended to destroy the virus, although from *Case* 25 we know that the virus can in some cases at least survive such a period of time. The negative result therefore in this case may be considered to be of some significance.

Similar remarks apply to *Case* 21. In this instance we know that the blood from which the filtrate was taken was infective on the date on which it was removed, as evidenced by the positive results obtained in *Cases* 16 *and* 17.

(*i*) *Cases in which a Pasteur-Chamberland Filtrate of the citrated blood was injected.*

Cases 1 to 5 received injections of a Pasteur-Chamberland filtrate of citrated blood taken on the third day of the disease and kept outside the body for three days before inoculation. In addition to this inoculation, Cases 2 to 5, four days later, each received a second inoculation of material from another dengue case. Cases 1, 2 and 5 remained perfectly well, Case 4 developed a typical attack of dengue eleven days after the first injection and seven days after the second injection. As detailed under "Consideration of Cases 1 to 9," we attributed the successful result in this case to the second inoculation and believe that the filtrate of citrated blood failed to convey infection. In Case 3 an illness developed twenty-two days after the first inoculation and eighteen days after the second inoculation. We are not prepared to say whether this disease was or was not an atypical form of dengue. If the disease was dengue infection could as reasonably be attributed to the second injection as to the first. It therefore appears that none of the five cases injected with the Pasteur-Chamberland filtrate from Natural Case A could with any reasonable certainty be considered as having received the infection from this source. This failure to convey the disease might result either because the blood from Natural Case A was not infective at the time it was withdrawn or that it lost its infectivity during transit and before inoculation, or that the filtration process separated the virus from the filtrate.

(*j*) *Cases shewing the presence of the virus in the blood on certain days of the disease.*

The presence of the virus on the second day of the disease is demonstrated by the results in Cases 12, 13, 16, 17 and 27.

That the virus is present on the third day of the disease is shewn in Cases 11, 25, 26 (32) and 29. If the positive results in Cases 6 and 7 are to be attributed to the first injections (*vide* Discussion on Cases 2 to 9), they also shew that blood is infective on the third day.

If the results in Cases 4 and 9 are to be attributed to the second injection (*vide* Discussion on Cases 2 to 9), then the infective material may still be present on the eighth day of the disease.

(*k*) *Case apparently shewing the absence of the virus after recovery from the disease.*

Case 31 received an injection of serum and corpuscles on the fourteenth day after the beginning of the illness of "B. B.": no symptoms followed. Unfortunately we were unable to inoculate this case later with an active virus so as to shew that he was not naturally immune.

(*l*) *Case shewing the establishment of Immunity shortly after recovery from an attack of Dengue Fever.*

Case 13 received a subcutaneous injection of serum and corpuscles on April 18th, 1916, became suddenly ill on April 25th, and passed through a typical attack of dengue. The temperature reached normal on April 29th but the rash had not completely faded until May 8th.

On June 2nd, 1916, he was reinjected subcutaneously with ·5 c.c. of serum and corpuscles from the case of "B. B." who contracted the disease through mosquito bites. *Case* 13 now became *Case* 30.

On the same date (June 2nd), a non-immune (Case 26–32) received an exactly similar injection from the case of "B. B." Case 30 remained unaffected as a result of his injection, while Case 26 (32) went through a typical attack of dengue beginning on June 11th. Though it must be borne in mind that it cannot be considered as established that the subcutaneous injection of infective blood from a case of dengue will certainly produce in a non-immune individual an attack of dengue fever, the results obtained in Case 30 point strongly to the view that his failure to develop the disease after an injection of serum and corpuscles known to be infective, was due to his having recently passed through a typical, though artificially produced, attack of the disease. The subcutaneous injection of the new infective material occurred forty-five days from the day when he received his first injection of infective material; thirty-eight days from the onset of his typical attack; thirty-five days from the time when his temperature practically reached normal

after this attack; and twenty-four days from the date on which the rash had disappeared and he was feeling well again—a period at which a definite measure of immunity may reasonably be considered to have been established.

From the results in Case 30 it may therefore be deduced, with the qualification referred to above, that an individual may be completely immune to the virus of dengue fever introduced subcutaneously after a period at least of twenty-four days, say one month, after complete recovery from a typical attack. One cannot say from this experiment that re-infection at an earlier period might not be effective. In other words a definite time may have to elapse before the establishment of any real immunity. We have no precise information on this point. Again how much longer after such an attack this complete immunity remains, is a subject for further investigation. This result is in accordance with the clinical experience of epidemic dengue, namely, that persons who have recovered from an attack are unlikely to suffer from a second attack during the epidemic in spite of the continuance of cases amongst non-immunes several months after these individuals had passed through their attack. There seems, however, some clinical evidence available that occasionally specific individuals may suffer from more than one attack of dengue during the prevalence of an epidemic. There seems little evidence to shew that such immunity exists for periods of time to be estimated in years, inasmuch as victims of one epidemic may be also victims of an epidemic occurring some years afterwards. The rarity, however, of an epidemic occurring in the same area in the immediately succeeding year suggests that some measure of immunity extends over this time. Case 30 would seem therefore to shew, as does clinical experience, that recovery from the disease is associated with an immunity to the disease which probably is the main factor in recovery, and that such recovery is not due of necessity to the organism having completed its life cycle in the human host, though still remaining in this host as a commensal parasite harmless to it but infective to the mosquito. Though it is possible that this may actually be the case, the introduction of further infective material, containing a virus which had not yet completed its possible cycle in the human host, failing in this case to convey the disease, and the clinical experience of immunity to the disease during an epidemic, both point to the view that such an immunity is established, and that it probably plays an important part in recovery from the disease.

(*m*) *Cases shewing that the blood can retain its infectivity outside the body for varying periods.*

For one to two days. In two of our positive cases (11, 32), the virus was outside the body less than one day. In two of the doubly injected positive cases it was also outside the body less than one day (4, 9). In four cases the virus was outside the body one day (12, 13, 16, 17). In one case two days (29). Thus in nine of our positive cases the virus was outside the body less than two days.

For four days. If the successful results in Cases 6 and 7 are to be attributed to the first injection (*vide* consideration of Cases 2 to 9), then the virus can exist without losing its infective properties for a period of four days outside the body at a mild early autumn temperature such as it was exposed to in transit from Murwillumbah to Sydney. Also in Case 27 the material was kept cool in an ice chest for four days before inoculation.

For seven days. In Case 25 the material was kept in an ice-chest for seven days before inoculation.

From the above we can conclude that the infective agent of dengue fever can survive in the blood outside the body for a period of four days and, sometimes at any rate, longer, viz., up to seven days.

Further work should be done upon this aspect.

(*n*) *Cases shewing the length of the incubation period of the inoculated disease.*

From 5 to 6 days.

Case 16. 5 days 20 hours.

Case 17. Under 6 days (to onset of fever; 7 days to onset of other symptoms).

Case 29. 4 days 21 hours to first symptoms; 6 days 8 hours to taking to bed.

From 6 to 7 days.

Case 9. 6 days 16 hours (if attack attributed to second injection; 11 days if attributed to first injection).

Case 12. 6 days 14 hours.

Case 13. 6 days 14 hours.

From 7 to 8 days.

Case 27. 7 days 21 hours.

From 8 to 9 days.

Case 6. 8 days 3 hours (if the disease were due to the first inoculation; just over 4 days if due to the second inoculation—*vide* Consideration of Cases 2 to 9).

Case 7. 7 days 20 hours (if the disease were due to the first inoculation; just over 4 days if due to the second inoculation—*vide* Consideration of Cases 2 to 9).

Case 11. 8 days 13 hours.

Case 25. 9 days.

Case 26 (32). 8 days 12 hours.

(*o*) *Is the length of the incubation period dependent on the strain of the virus, or on the susceptibility of the patient or on both?*

We have found by the inoculation of blood and by our mosquito-fed cases that the incubation period of the disease may vary from a little over five days to nine days. The question arises as to why such a variation exists. It is well known in most diseases that such a variation of several days between the shortest known incubation period and the longest known incubation period does exist.

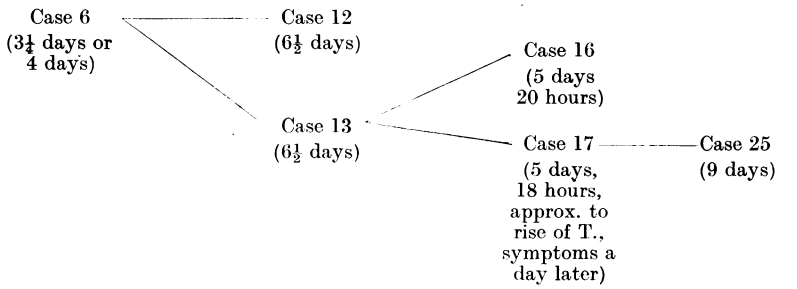
Are such variations due to mutational differences in the virus, to greater or less resistance in the patient, or to differences in the amount of infective materially originally received?

It can be at once understood that a dose of the infecting organism not much above the minimum might result in a prolonged incubation period owing to the longer period perhaps required for the organism to multiply sufficiently to produce symptoms and signs.

Provided, however, that the dose is a reasonably large one, is there any evidence to show that the incubation period will vary with the virus or with the susceptibility of the patient?

The results in Cases 12 and 13 and in Cases 16 and 17 would seem to suggest that the same virus in a sufficient dose tends to produce a disease with approximately the same incubation period. Case 12 received a Pasteur-Chamberland filtrate of clot and serum from blood taken on the second day of an attack of dengue (Case 6), whilst Case 13 received the untreated serum and corpuscles of the same blood. Both were inoculated at the same time, and both developed the disease contemporaneously six and a half days later. In Case 16 the volunteer received an inoculation of washed corpuscles, and in Case 17 the plasma in citrate normal saline solution, the blood in both cases being derived from the same patient (Case 13). The two cases received their inoculations within fifteen minutes of each other, and Case 16 developed dengue fever five days and twenty hours later, whilst Case 17 developed the disease, as indicated by a rise of temperature alone, apparently a few hours earlier (the exact time has not been noted). Case 25 inoculated

from Case 17 had an incubation period of nine days. This sequence of cases can be graphically represented as follows:



An examination of this series of cases seems to shew that the same virus during its passage through a series of individuals may produce illnesses with varying incubation periods of from six and a half, possibly four days, to nine days, but that if the virus be taken at any particular moment and injected into two individuals, it may result in practically identical incubation periods. In other words it would seem that the length of the incubation period is determined more by the state of the virus than by the state of the patient. It should be noted further that the menstruum in which the virus was obtained varied somewhat in the individuals of each pair, so that presumably different doses of the virus were received by the individuals of each pair.

In considering these results, however, due consideration must be given to the fact that only two instances of equal incubation periods are dealt with, and that in Case 17 the temperature rose nearly a day before any symptoms were manifested so that the early incidence of the disease would have been overlooked had the temperature not been taken. The results, however, indicate that further work might very well be carried out to ascertain whether the hypothesis suggested is one of general applicability or not.

(*p*) *Case Sequences in Relation to Immunity.*

Under this heading are included those instances in which the particular virus has been passed in succession by inoculation from one individual to a second, from the second to a third, and so on. The following is an instance of such successful sub-inoculations.

In Case 6 the virus was present on the second day of the disease as proved by successful sub-inoculations into Cases 12 and 13. From Case 13 further successful sub-inoculations were made from material

taken on the second day of the disease and injected into Cases 16 and 17. From material taken from Case 17 on the third day of the disease a further successful sub-inoculation was made into Case 25. In this particular series we have been successful in conveying the disease by inoculation and sub-inoculation consecutively into four individuals. The virus from which Case 6 was inoculated was presumably obtained from the blood of Case "B" on the third day of the natural disease (possibly from that of Case "C" on the eighth day); thereafter in Cases 13 and 17 the virus was obtained from bloods taken on the second and third days respectively of the inoculated disease, and in Case 25 from blood taken on the third day. We thus see that this virus, by the time it reached Case 25, had produced in human beings the following days of disease, viz:, 3 + 2 + 3 + 3, without the virus having passed through any stage of its life history in the intermediate host, the mosquito. When it reached Case 25 it was capable of producing a disease in this patient lasting five days, followed nine days later by a relapse lasting another five days, followed nine days later by a relapse lasting another five days. In other words this virus produced in human beings, without going through any phase in the mosquito, sixteen days of fever followed by a relapse of five days of fever. It may be further noted that the disease in Case 25—the end of the series—was as pronounced as in the first case of the series, shewing that there had been no definite attenuation of the virus. Between the various inoculations, this virus had been outside the human body for four days (presumably) before inoculation into Case 6; for one day between Cases 6 and 13; for one day between Cases 13 and 17; and for eight days between Cases 17 and 25; that is to say, that during the period covered by the sixteen days of fever, the virus itself had been outside the human body for a period of fourteen days. The incubation periods of the disease in the four cases forming the series are respectively eight, six and a half, six and nine, making a total of twenty-nine and a half days. We now get the following totals in connection with this virus when inoculated in series, viz., sixteen days of fever, fourteen days outside the body, and twenty-nine and a half days incubating in the body before manifesting the disease. The total number of days obtained by adding these together is fifty-nine and a half, whereas we find that the virus left the original case on April 4th on the third day of the disease and had completed the primary attack of dengue in Case 25 on May 26th, giving a total of only fifty-five days. The discrepancy is easily explained, inasmuch as incomplete days have been taken as full days in estimating the period outside the body,

the days of the disease, and so on. As a matter of fact, therefore, the figures sixteen, fourteen and twenty-nine and a half, more particularly the two former, are each higher than they should be, probably by a day or a little more. The relative proportions, however, still remain. We thus find that in our series in which the virus was passed from individual to individual over a period of fifty-five days, roughly one-fourth of these days was spent outside the human body, a second quarter was occupied by the virus in producing manifest disease, and in about half of them the virus was incubating in the human body. What information can be gathered from these results? The natural disease produces in its victim an illness definitely lasting from five to seven days followed by convalescence. Does the fever end after the five to seven days of illness because the virus has gone through a phase of development and has now reached a stage ready for transmission to another (invertebrate) host but no longer capable of producing a reaction in the human host; or has the fever subsided because the human host has been able to combat successfully and overcome the virus?

Apart from the question as to whether dengue is usually only infective for the mosquito or by inoculation to other human beings during the first three days of the disease (this point is still undecided), the results obtained in this series would seem to indicate that the latter and not the former view is the correct one. If the virus requires seven days to complete its phase of development in the human body, then sub-inoculations in a series of individuals should fail at the end of an aggregate of seven days fever, whereas in our series we were able to produce sixteen days of fever followed by a relapse. The objection of course may be raised that withdrawal of the blood and keeping it for varying periods of time outside the body, associated with the necessary incubation periods, so interfere with the maturation of the virus in the human body, that a considerably longer period might be required for it thus to mature than would be the case could complete maturation occur in one individual. The balance of evidence, however, seems to be in favour of the view that the patient recovers from his attack of dengue because of his protective reaction against the virus rather than that the virus, having passed through and completed a phase of its existence (viz., that productive of disease in man), still remained in the human host in a stage incapable of infecting human beings with disease (= gamete formation in malarial organisms).

The accompanying Chart I represents graphically the history of this virus. The horizontal lines indicate in days the presence of the

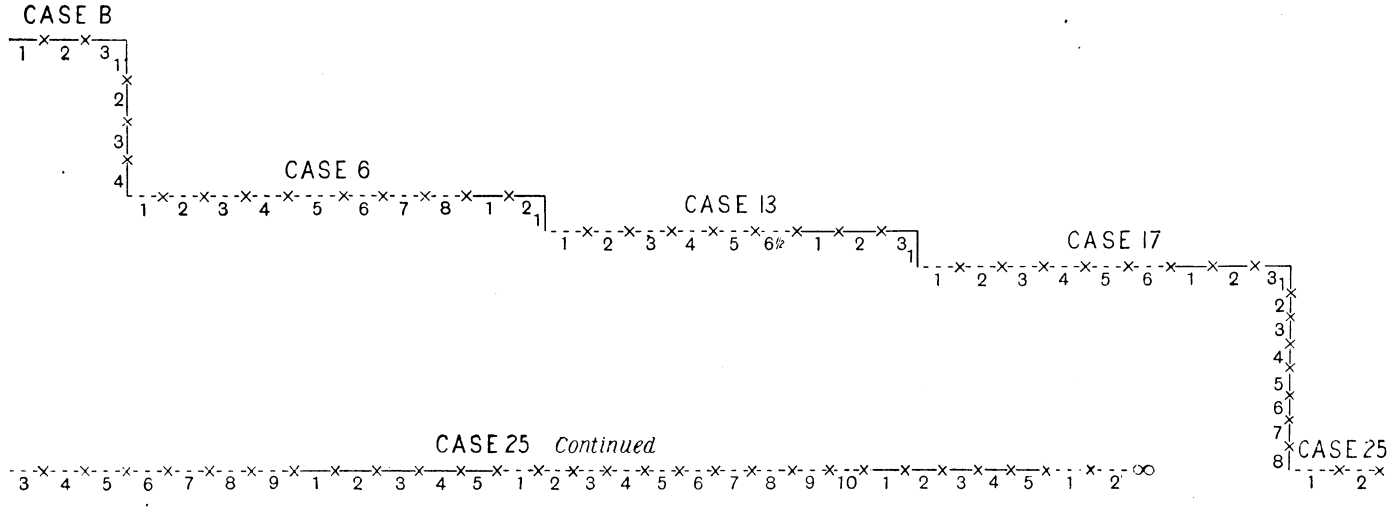


Chart I. Illustrating case sequences as described in the text.

virus in human beings, whilst the vertical lines indicate similarly the presence of the virus outside the human body *in vitro*. Days marked thus — indicate that on these dates the virus was causing disease in the human victim, whilst days marked thus - - - indicate that it was incubating or had ceased to manifest its presence unmistakeably in the human case.

(*q*) *Can the disease be conveyed by an application of serum to a scarification?*

In Case 22 the arm of the volunteer was scarified as for an ordinary vaccination with calf lymph, and the mixed sera from Cases 16 and 17 were rubbed into the scarified area. The sera were obtained from blood from these cases taken on the third days of the inoculated diseases and at least in Case 17 we know, by the successful inoculation of Case 25, that the virus was present in the material. The result in this case was very doubtful. The patient shewed occasional slight signs and symptoms and a slight rise of temperature, which may have been due to his passing through an exceedingly mild attack of dengue fever. Such a result might perhaps be anticipated if a very mild dose of the virus gained entrance to the human host.

(*r*) *Can the disease be conveyed by an application of infective material to the nostrils?*

In Case 23 the nostrils were swabbed with the mixed sera of Cases 16 and 17 obtained from blood taken on the third days of the inoculated diseases, which we know, in the case of Case 17 at least, to be infective as proved by the successful inoculation of Case 25. The result must be considered as negative, though some very slight symptoms were manifested which may possibly have some significance.

Can the virus be conveyed by the ingestion of infective blood?

As various infectious diseases have been thought at times to be possible of conveyance by the ingestion of infective material, it was thought of interest to see whether gargling the throat and then swallowing a neutral mixture containing infective dengue blood would result in an attack of dengue or not. If in such cases the infection occurred through the pharynx, gargling would facilitate the entrance of the organism, whereas if infection occurred through the stomach or lower down the alimentary canal, swallowing the blood should achieve the

result sought. In Case 24 the mixed sera of Cases 16 and 17, which we know contained the virus, were added to a neutral mixture, and the throat gargled with this, and the material then swallowed. He developed a doubtful but rather suggestive illness, and though his case cannot be considered as being definitely one of dengue, it is nevertheless very suggestive of a mild attack.

In Case 26 blood was similarly used as a gargle and then swallowed, but this blood had been taken from Case 25 on the fourth day of the inoculated disease, and we have no proof by means of other inoculations that it was at this period infective. Case 26 did not develop within the ordinary incubation period any symptoms definitely suggestive of an attack of dengue. In this case the result is somewhat obscured by his receiving subsequently a subcutaneous inoculation of infective material which did produce a typical attack of dengue. It therefore seems clear that at any rate the gargling and the swallowing of the blood did not produce any protective bodies which prevented the patient developing a typical attack by subsequent inoculation of infective material.

(*s*) *The Relationship of Dengue to Yellow Fever.*

The text-books on Tropical Medicine dwell on the differential diagnosis of dengue and yellow fever which may co-exist in places. Neither of us has seen a case of yellow fever and so we are handicapped in considering this aspect of the question. From descriptions, however, it appears that a mild attack of yellow fever may be hard to differentiate from an attack of dengue in the absence of a rash. Castellani and Chalmers state that yellow fever can be differentiated from dengue by its slower pulse, jaundice and haematemesis. From our experience of the clinical disease and of inoculated cases, we have found that a pulse relatively and absolutely slow may occur in cases of dengue, and that therefore this point may not be of material help in a doubtful case. The occurrence of jaundice would be of considerable importance, but we have heard of occasional instances of slight jaundice occurring in dengue patients during the recent epidemic. We have not heard of any cases of haematemesis, but have noted that vomiting has been a sign in some cases of the disease.

We find that the incubation period of the mosquito-conveyed disease and of the inoculated disease in dengue varies from about five to nine days. In yellow fever the incubation period is said to vary from two days twenty-two hours to six days two hours.

We find that the virus of both yellow fever and dengue is transmitted by the same mosquito, *Stegomyia fasciata*.

This shows that there are strong points of resemblance between dengue and yellow fever; and slight, but definite, points of difference. Dengue usually has a definite rash—yellow fever has none. Jaundice and haematemesis are characteristic of yellow fever. The incubation period of dengue is slightly longer than that of yellow fever.

Summary of Results.

1. Dengue Fever in Australia is undoubtedly an introduced disease. It has been existent from time to time in epidemic form since 1885.

2. The clinical description of the disease agrees with that of the Dengue described in text-books, the only departure noted being the distinct tendency to a relatively, and sometimes absolutely, slow pulse rate as compared with the temperature.

3. It is possible that under the single term "Dengue" more than one disease is at present included.

4. Epidemic Dengue in Australia is approximately co-extensive with the known distribution of *Stegomyia fasciata*. It does not extend beyond the area in which this mosquito is prevalent.

5. *Stegomyia fasciata* mosquitoes caught in a dengue infected district in the surroundings of cases of the disease, and some of them known to have fed on a dengue patient on the first and second days of his illness, transported to a non-dengue district, reproduced the disease in four out of seven persons on whom biting experiments were conducted.

6. Blood taken from three of these four cases reproduced the disease when injected into further persons. The blood of one case was not tested.

7. The incubation period of the four cases was found to be possibly between five and nine and a half days, probably between six and a half and nine and a half days, counting from the bitings to the definite onsets.

8. No known case of contagion occurred from any of the above four cases.

9. No evidence was obtained from two cases, one of which was heavily and repeatedly bitten with *Culex fatigans*, that *Culex fatigans* is capable of acting as a transmitter of dengue fever.

10. The blood of patients suffering from an attack of dengue can reproduce the disease when inoculated subcutaneously into healthy persons.

11. The disease thus inoculated is typical in every way of dengue fever naturally contracted. The inoculated disease may or may not shew marked skin rashes and double phases in the temperature charts, and presents a relatively and sometimes absolutely slow pulse; such variations occur in the natural disease. The incubation period of the inoculated disease varies from five to nine days corresponding with the incubation period of the mosquito-transmitted disease.

12. Results of the inoculations shew that:

(*a*) The virus of dengue is present in the blood as a whole.

(*b*) The serum of clotted infective blood may contain the virus.

(*c*) With washed corpuscles one apparently positive result was obtained out of three experiments.

(*d*) The fluid part of citrated infective blood may contain the virus.

(*e*) With Pasteur-Chamberland filtrates of infected serum and corpuscles, one positive result was obtained out of five experiments.

In considering these results failure to convey the disease must not necessarily be interpreted as meaning that the menstruum employed never does contain the virus, as in some of the cases the blood may no longer have been infective at the time at which it was withdrawn.

(*f*) The presence of the virus in the blood has been demonstrated on the second and third days of the disease. Two experiments made may possibly be interpreted as shewing that infective material may still be present on the eighth day of the disease.

(*g*) One experiment appears to indicate that the virus is no longer present in the blood on the fourteenth day from the beginning of the illness.

(*h*) Immunity to the inoculation of infective blood appears to be complete twenty-four days after recovery from a typical attack of dengue.

(*i*) Infected blood may maintain its infectivity outside the body if kept in a cool place for seven days at least.

(*j*) In two instances two individuals inoculated with the same material on the same day exhibited incubation periods practically identical in duration.

(*k*) The infection of dengue can be conveyed by sub-inoculations from individual to individual at least to the fourth generation without the resultant disease departing from the type of the natural disease.

(*l*) The disease has not been conveyed by the application of infective serum to a scarified area; nor apparently has it been conveyed by the application of infective material by swabbing to the nostrils.

(*m*) A very doubtful and probably negative result followed the gargling of the throat with infective material followed by swallowing of the same.

(*n*) Dengue fever has close analogies with yellow fever.

Future Investigations.

The following points require elucidation by further research and we trust that later we may have an opportunity of doing this:

1. To ascertain the period that must elapse after *Stegomyia fasciata* has bitten a dengue patient before the insect can transmit the disease to another human being.
2. To ascertain the length of time that such an infected mosquito may remain infective.
3. To ascertain whether the virus can be transmitted through the eggs to the progeny of such infected mosquitoes.
4. To ascertain whether *Culex fatigans*, *Scutomyia notoscripta*, or any other mosquito can also act as intermediate hosts of the organism of dengue.
5. To ascertain for how long after the third day of the disease the virus may still exist in the blood of the patient.
6. To ascertain how long immunity after an attack may last.
7. A repetition of the experiments suggesting that the virus may be able to pass through a Pasteur-Chamberland filter.
8. A repetition of the experiments with washed corpuscles to ascertain whether the organism exists as an intra-corpuscular parasite or merely becomes attached to the corpuscles.
9. A repetition of the experiments with serum to ascertain whether the positive results obtained from this source were due to accidental inclusion of infected corpuscles, or liberation of parasites into the serum from injured corpuscles, or whether these results were due to the virus being a natural inhabitant of the serum.
10. A repetition of the experiments with ingested blood and with the application of infected material to the nares and to local scarified areas to ascertain whether the virus so ingested or applied can induce the disease.

APPENDIX I.

DETAILS OF NATURAL CASES OF DENGUE FROM WHOM INOCULATIONS WERE MADE.

Case A. "*Mr P.*" *Murwillumbah.* He first became ill on 3. iv. 16 and was in the midst of a typical attack of dengue with a temperature of 102° F. when blood was taken from him at 11 a.m. on 5. iv. A portion of this blood was injected into citrate normal saline solution, and part was allowed to clot and the serum then separated and sealed. The Wassermann reaction applied to an inactivated portion of the serum proved negative. Owing to contamination the separated serum could not be used for inoculation purposes on arrival in Sydney. A Pasteur-Chamberland filtrate from the citrated blood was used for inoculation purposes in Cases 1 to 5. Cases 1, 2 and 5 remained well. Cases 2 to 5 also received a second inoculation of material from Natural Case C four days after the first injection. Case 3 developed an illness of doubtful nature beginning 22 days after the first injection and 18 days after the second. Case 4 developed an attack of dengue 11 days after the first injection and 7 days after the second. As indicated in our summary of Cases 2 to 9, each of which received two injections, we are inclined to consider that the infection in Case 4 was derived from the second injection and not from the first. This view is chiefly founded on the length of the incubation period.

Case B. "*Mr H.*" *Murwillumbah.* This patient was taken ill on 2. iv. 16. He had pain in the back and down the legs and felt "squeamish" at times. He had had no vomiting. When seen on 4. iv. he was in the midst of a typical attack of dengue with a temperature of 102° F. One eye was congested. His wife at this time was also ill, her attack of dengue having begun on 30. iii. with pain in the back; she also vomited up her dinner. On 31. iii. she felt very sick and had pains in the back and down the backs of the legs, shooting in character, and a sore throat. On 1. iv. she was very sick, and had a rash. When seen on 3. iv. her tongue was clean, with a temperature of 98·4° F. She was covered with a punctate, scarletiniform rash, her hands being also covered with a marked rash. Blood was taken from the husband on 4. iv. and divided into three portions, one being injected into citrate normal saline solution, a second being allowed to clot when the serum was separated and sealed in a tube, whilst the Wassermann test was applied to an inactivated portion of serum with a negative result. The clotted blood from which the serum had been extracted was also kept.

On arrival in Sydney, the citrated blood was found to be contaminated. Cases 6 and 7 received injections from the mixed serum and clot, whilst Cases 8 and 9 received injections of the clear serum. These four cases four days later also received injections of material from Natural Case C. Cases 6 and 7 developed attacks of dengue eight days after the first injection and four days after the second. As indicated under our review of Cases 2 to 9, we attribute infection to the first inoculation, that is, to material from Natural Case B. Case 8 remained perfectly well, but Case 9 developed an attack of dengue eleven days after the first injection and seven days after the second injection. In this case we are inclined to attribute the infection to the second inoculation.

Case C. "*E.S.*" was a patient who had left a dengue district to enter the Coast Hospital, Sydney, to undergo an operation. On arrival at the Coast Hospital he was found to be recovering from an attack of dengue which had been contracted in the endemic area. The history of his movements prior to arrival at the hospital is as follows: He left his home at Tyalgum at 9 a.m. on 31. iii. and arrived at Murwillumbah at 1 p.m. the same day, and had dinner and stayed the night at a boarding-house, leaving by the 6.20 a.m. train for Lismore on 1. iv. He had dinner at Lismore and left for Coraki at 2 p.m. by boat, arriving there at 5 p.m. He stayed at a boarding-house in Coraki from the afternoon of the 1st until the 4th April, when at 7.30 p.m. he left by boat for Sydney. On 4. iv, before leaving Coraki, he had a nasty languid feeling which he could not understand. To get over this feeling he went for a long row in the afternoon, and felt well whilst taking this exercise, but as soon as he got back and became cool again the same languid feeling recurred. He could not account for this feeling until about 9 p.m. the same night on board the steamer at sea, when his eyes began to burn and his bones began to ache. He gradually grew worse until he reached Sydney about 2 p.m. on 6. iv. He stayed at the People's Palace in Sydney until admitted to the Coast Hospital at about 3 p.m. on 10. iv, when he felt in a much improved condition, but was a week in bed at the hospital. He states that whilst at the boarding-house at Murwillumbah he was bitten on the back of the left wrist by a mosquito, and that there was also a dengue patient sleeping in the next room.

On 11. iv. blood was taken from this patient—portion was placed in citrate normal saline solution and portion was allowed to clot and the serum separated. Next day Cases 2, 3, 4, 5, 7 and 8 received injections of the citrated blood, and Cases 6 and 9 of the serum. All these cases had four days previously received injections of material from Natural Cases A or B. Of the cases injected with citrated blood, Case 3 developed a doubtful illness twenty-two days after the first injection and eighteen days after the second injection. Case 4 developed dengue eleven days after the first injection and seven days after the second injection, and Case 7 an attack of dengue eight days after the first injection and four days after the second injection. Cases 2, 5 and 8 all remained negative. As detailed under the consideration of Cases 2 to 9 we attribute the infection of Case 7 to the first injection, and the infection of Case 4 to the second injection, namely, the material from Natural Case C. Cases 6 and 9 injected with the clear serum both developed dengue—the first, eight days after the first injection and four days after the second, and Case 9, eleven days after the first injection and seven days after the second. Here again we attribute the infection of Case 6 to the first injection and Case 9 to the second injection.

Case D. This patient was a soldier who was found suffering from an attack of dengue in camp at Sydney. He had been in camp in a dengue district (Brisbane) for some time. He had spent the previous Saturday, 15. iv. 16, at Sandgate near Brisbane, where he says there were enormous numbers of mosquitoes. He remained well till the evening when he felt "off-colour." Next day he had pains in the back and across the loins and had a severe headache especially behind the ears. He says he "saw double." There was eye pain on movement. There was no running at the nose; the throat was dry but not sore. He was ill on Monday, 17. iv, and entrained for Sydney on Tuesday. In the train his temperature was 102° F. During

the train journey he first noticed a rash on the chest on the Wednesday morning. He vomited in the train very severely, the material being pale coloured. He arrived at Sydney on Wednesday night. He was admitted to hospital at 9.30 p.m. the same evening with a temperature on 99·8° F. and a well-marked rash over the chest and back. Next day the temperature was 104°. On Friday, 21. iv, the face was flushed and the eyes injected and he looked ill though he said he was getting better. He still had pains in the back, etc., the eyes were injected and painful, and he was shivering. On enquiry, he stated that he had had dengue twice previously, but not during the present epidemic. On 21. iv. blood was taken from this patient. It was kept on ice till 25. iv. To a portion a Wassermann test was applied with a negative result. The clot and serum was diluted with normal saline solution and passed through a Pasteur-Chamberland filter. Before passing through the filter the material was inoculated with *B. prodigiosus*. Cultures made after filtration proved negative to ordinary bacteria. This material was injected on 28. iv. 16 into Case 18 with a negative result.

Case E. "*J.B.C.*" (*one of us*). He reached the outskirts of the dengue area at Byron Bay at 7 a.m. on 3. iv. 16. At 10.30 a.m. he reached Murwillumbah where the epidemic was still severe, though apparently on the decline. During the rest of this day and on April 4th and 5th he lived in an hotel in the centre of the dengue area, and saw a number of cases of the disease, and caught a number of mosquitoes, both *Stegomyia fasciata* and *Culex fatigans*, in the surroundings of the patients. By means of mosquito netting and citronella oil and other devices, he protected himself as far as possible from being bitten by day- or night-biting mosquitoes. On one or two occasions in patients' rooms *S. fasciata* settled on his arm or face and began inserting their proboscides. These mosquitoes were immediately captured in test tubes. As he is not particularly sensitive to mosquito bites he may have been bitten unawares by other mosquitoes. Though he slept under mosquito curtains at night time, and though he did not find any mosquitoes in the net next morning, he cannot be certain that he was not bitten by such during the night. At about 5.30 on the afternoon of April 5th, he found that the unfed *Stegomyia* in his mosquito cage were escaping through the meshes of the wire, which were hardly close enough to prevent a slender mosquito from wriggling through. As the guinea-pig which had been taken up for the mosquitoes in the cages to feed upon, was injuring the insects and tending to drive the thin ones through the wire meshing, he could not use this animal for distending the bellies of the mosquitoes, and consequently inserted his own hand and forearm to stop the exodus. The *Stegomyia* at once settled upon it and eight or ten at least engorged themselves. These mosquitoes had been collected from houses in which cases of dengue had occurred, and in a number of instances actually from the rooms inhabited by dengue patients. At about 10 p.m. that same night in the dark he inserted his hand into the box containing *C. fatigans*. He left it there motionless for about a quarter of an hour. He did not feel the bites of any mosquitoes, but is relatively insensitive to the bites of this insect, which may therefore have bitten him considerably. On 6. iv. 16 he descended the Tweed River to the Tweed Heads where he saw further cases of dengue and caught more mosquitoes. He reached Brisbane that night, and thinks he may have been bitten by *C. fatigans* but cannot be certain; he left Brisbane at 8 o'clock on the 7th and, with it, the dengue area,

arriving at Sydney at about 11 o'clock on the morning of the 8th with his two cages of mosquitoes.

On arrival, another of us ("B.B."), who had not been to the dengue area at that time, placed his hand in the box containing *Stegomyia*, but for some reason these would not bite him. "J.B.C." then inserted his hand and a *Stegomyia* at once settled upon it and began to pierce the skin. The insect was shaken off and the hand withdrawn.

"J.B.C." remained well until 12. iv. 16 and also on that morning when arising. Later in the morning he felt perhaps very slight and indefinite malaise. A similar condition existed after lunch with a very slight tired feeling. At 5.15 p.m. the tired feeling was more definite, accompanied by the merest trace of headache and a feeling of discomfort in the eyeballs. The tired feeling resembled that of a cold, but with no coryza. His temperature at 6 p.m. was 99·2° F.; at 7.30, 100°; and at 9 p.m. 101·2°, with pulse 102 and respirations 18. He still only felt a tired feeling in the back and the legs with slight giddiness. He spent a very restless night—one of the most restless that he has ever experienced. He kept dropping off to sleep and sleeping for short intervals, and then would awaken suddenly with acute mental alertness. He was unable to get comfortable in any position, and had a slight headache and very slight sore throat, and he sneezed two or three times.

13. iv. At 6 a.m., temperature 98·4° F. A very tired feeling with indefinite pain in the back, legs, and eyeballs. 7.45 a.m., temp. 98·4°. Went into town at 8.15 a.m. At 11 a.m., temp. 100·2°; returned home feeling indefinitely ill; at 2 p.m., temp. 100·8°; slept fairly well in a deck chair from 2 till 4 p.m. At 6.30, temp. 101°, pulse 94; the eyes congested, headache slighter; at 9 p.m., temp. 101·5°; pulse 92.

14. iv. Passed a fairly good night. At 6.45 a.m., temp. 100·2°, slight subcuticular mottling of the abdomen. At 8 a.m. temp. 100°, pulse 84. At noon, temp. 100·2°; at 6.15 p.m., temp. 100·5°, pulse 80; at 9.45 p.m., temp. 100°, pulse 76.

15. iv. Passed a good night. Temp. at 7 a.m., 98·8°; at 3.30 p.m., 99·4°, pulse 80; feeling nearly well; weeded a little in the garden sitting down. At 6.45 p.m., temp. 99·6°, pulse 80, feeling perhaps a trifle more tired than on the previous evening; a bitter taste in the mouth. At 10 p.m., temp. 99·2°.

16. iv. Passed a good night. At 7.30 a.m., temp. 98·6°, feeling stiff; at 2 p.m., temp. 99·2°; at 6 p.m., temp. 100°; at 10 p.m., temp. 99·8°.

17. iv. Temperature at 7 a.m., 99·4°; 1.30 p.m., 100·4°; 6 p.m., 100°; 10 p.m., 100·8°.

18. iv. Temperature at 7 a.m., 99°.

Up to this time "J.B.C." had not "felt himself" since his illness began, but when he awakened on the morning of 19. iv. he experienced his usual feeling of health which was quite different from the feeling on arising the day before, even though there was nothing tangible to be recognised beyond a very slight increase in temperature. Thereafter for several days he felt a certain amount of stiffness of the muscles and of aching during movements of the eyeballs. Also, during his convalescence, he took a dislike to tea and to smoking for a few days. These dislikes fortunately soon disappeared.

Blood was taken from this case on 14. iv. 16 and portion was injected into citrate normal saline solution, and part was allowed to clot and the serum abstracted. On the same day Case 10 was injected with the corpuscles from the citrated blood after

thorough washing, and no illness resulted. Case 11 received an injection of the serum, and eight and a half days later developed a typical attack of dengue with a typical, almost morbilliform, rash. Such a typical rash had been absent during the illness of Case E. Cases 14 and 15 received sub-injections from the successful Case 11 with material taken on the fourth day of the disease and received into citrate normal saline solution. One case received the washed corpuscles, and the other case the citrated plasma, but neither became ill.

APPENDIX II.

DETAILS OF FIRST SERIES OF MOSQUITO EXPERIMENTS.

A. EXPERIMENTS WITH *STEGOMYIA FASCIATA*.

At Murwillumbah on April 3rd, 4th, and 5th a number of *S. fasciata* were caught in the rooms of persons suffering from dengue or on the mosquito curtains of their beds, a few being also captured in houses where cases of dengue had recently occurred. These were supplemented by a few further mosquitoes caught on April 6th at Tweed Heads in the rooms of dengue patients. These mosquitoes were contained in a chocolate box with a wire gauze front and a sleeve of mosquito netting leading to an opening on one side. A small vessel with water was placed at the bottom of the cage. A guinea-pig was taken with us for the purpose of allowing the mosquitoes to feed upon it, but owing to the smallness of the cage and the hairy coat of the animal, attempts to use it for feeding purposes were not successful and caused damage to a number of the mosquitoes, so it was dispensed with. The wire gauze was unfortunately not of a fine enough mesh to prevent a slender *Stegomyia* from occasionally wriggling through. As a number were escaping in this way, on April 5th at Murwillumbah at about 5.30 p.m. one of us (J.B.C.) inserted his arm into the cage when eight or ten out of about forty mosquitoes in the cage settled on his hand. These could be seen distending themselves with blood, and yet no sensation of pain or discomfort was at any time felt. On April 6th at Tweed Heads two dengue patients, one a sailor with a high temperature, and the other a Kanaka, placed their hands in the cage and several mosquitoes at least bit each individual. From Tweed Heads the mosquitoes were taken to Brisbane and thence to Sydney, which was reached on April 8th about 11 a.m. Thereafter the following persons were bitten by these mosquitoes in Sydney:

April 8th, 11 a.m.: One of us (B. B.) placed his hand in the cage; one or two mosquitoes apparently bit him but for some reason they would not feed freely. The other of us (J. B. C.) was accidentally bitten by one mosquito at the same time. The Assistant (W. T.) who accompanied one of us was also bitten by one of the mosquitoes, but this was barely allowed to draw blood. "J. G.," Laboratory Assistant to one of us who had volunteered for these experiments, was bitten by eight mosquitoes at 12.45 p.m. At 7 p.m. one of us (B. B.) was bitten by one of the mosquitoes.

April 9th: About twenty mosquitoes alive. "B. B." bitten by one in the morning.

April 10th: Owing to the shaking of a motor bicycle a number of mosquitoes were on this day unfortunately injured, only three remaining alive. One of these bit "B. B." in the morning. The volunteer "J. G." could not induce any of the three to bite him at 5 p.m.

April 11th: "J. G." bitten by two mosquitoes at 9.15 a.m.

April 12th: "J. G." bitten by two mosquitoes at 9.15 a.m.

April 13th: Mosquitoes would not bite "J. G." Two still alive.

Of the four persons bitten by this batch of mosquitoes, one (J. B. C.) developed a mild attack of dengue on the afternoon of April 12th. It seems probable that the disease was contracted by the mosquitoes which fed upon him at 5.30 p.m. on April 5th at Murwillumbah. "B. B.," "W. T.," and "J. G." developed no symptoms indicative of dengue fever at this time.

B. Experiments with *Culex fatigans*.

A number of *C. fatigans* were collected at Murwillumbah on April 3rd, 4th and 5th and at Tweed Heads on April 6th. These were all caught in rooms inhabited by dengue patients, either on the walls or on the mosquito netting. They were kept in a chocolate box with gauze wire front, a small vessel of water being placed at the bottom during the night time in which the eggs could be laid. At 10 p.m. on April 5th at Murwillumbah, and on April 6th at Brisbane, one of us (J. B. C.) inserted his hand into the cage for about twenty minutes. On neither occasion were any bites felt, but the bite of this mosquito is often not felt by the individual attacked. The mosquitoes reached Sydney on April 8th. They were taken to the Coast Hospital where a volunteer ("— McC.") placed his hand in the box in the dark at 7.30 p.m. Only about eight mosquitoes were alive in the cage and the volunteer thought that four of these bit him. At 9 p.m. one of our Assistants ("J. O. S.") put his hand into the cage and was, he thinks, bitten by two of the mosquitoes.

April 9th: "— McC" inserted his hand again at 7.30 p.m. but felt no definite bites though the mosquitoes settled on his hand. Several bit "J. O. S." at 9 p.m.

April 11th: "— McC." at 7.30 p.m. placed his hand in the box. Apparently none of the mosquitoes bit. "J. O. S." at 9 p.m. was bitten by one mosquito with certainty.

Thereafter neither of these individuals showed any signs indicative of dengue.

APPENDIX III.

DETAILED HISTORIES OF THE FOUR SUCCESSFUL CASES IN WHICH THE VIRUS OF DENGUE WAS CONVEYED BY *STEGOMYIA FASCIATA* IN THE SECOND SERIES OF MOSQUITO EXPERIMENTS.

Case I. *J. G.*, aet. 18, male, laboratory assistant. Not previously in a dengue district. Subject of unsuccessful biting experiments (*Stegomyia*) on 8. iv, 11. iv, and 12. iv.

11. v. 16. Bitten by some twenty-eight *Stegomyia* at 2.15 p.m.

19. v. Quite well in the morning. He felt a slight headache first in the afternoon. He came into town at about 7 p.m., to be bitten by mosquitoes, and while sitting with his hand in the cage noticed a feeling of heat and that his headache was worse. He went to lecture after this, and had to go out of the room and go home. He had no evening meal, and went to bed feeling shivery and hot at the same time, and spent a restless night. No vomiting occurred. Incubation period, eight days five hours.

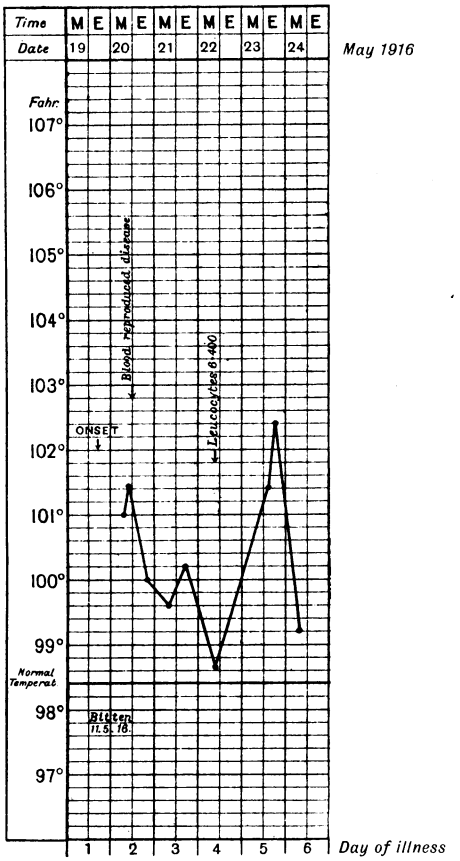


Chart II. Mosquito Case I. J. G.

20. v. He came in to work. One of us (B. B.) saw that he was ill, and that he had a typical dengue face, swollen, hot, and purplish in colour. The headache was severe in the frontal region. His eyes felt as if they were "being pulled," and hurt somewhat to move. There was pain in the neck, and abdominal pains early in the morning. A weak feeling, as in influenza, but no definite pains were complained of elsewhere. There was no coryza, but the eyes were injected. Anorexia was marked, but no nausea complained of. The tongue was furred, creamy at the back, and the

tip typically strawberry. There were three motions since rising. Temperature 101° F. at 9 a.m.; 101·5° at 11 a.m.; pulse-rate 105—see chart. We noted an indefinite subcuticular mottling. Blood was taken for injection experiments. The Wassermann test was negative. The patient was sent home too ill to work.

21. v. Stopped at home.

22. v. He came into town. There was doubtful mottling on the back and forearms. The tongue was still furred, with strawberry tip and edge. His legs were very painful. We took blood for injection experiment. The patient said that he felt "pretty well." Blood examination: leucocytes, 6400; polymorphonuclears, 78 per cent.; mononuclears, 22 per cent. (100 cells counted). No parasites were found in the blood.

23. v. Temperature at 7 a.m. was 102·2° F. He felt worse, the head and eyes were bad. There was an indefinite mottled rash on the chest, arms and back. Temperature at 4 p.m. 101·4°, pulse rate 120. The face was flushed and he looked sick. No obvious coryza was noted.

24. v. The temperature at 7.30 a.m. was 99·2° F. The head was not aching, and the eyes were better. The back was stiff on waking and on bending. He felt fairly well. A definite, slight, mottled rash was seen on the back, lumbar region, and abdomen. It may be described as "midway between measles and scarlet fever, only much less marked."

After this the patient was well, and continued to work as usual.

One c.cm. of the blood was taken from this patient on 20. v, and a volunteer (H. K., Case 27) was injected on 24. v. 16, and became ill eight days later (1. vi, mid-day), and had a typical attack of dengue, with a double temperature curve, typical symptoms and slow pulse.

One c.cm. of the blood was also taken from this patient on 22. v, and was injected into a volunteer (N. McA., Case 28) on 24. v, but no definite attack of dengue developed. This patient's temperature had a definite tendency to be above normal from the start, several times rising to just over 99° F., and on 8. vi, the afternoon temperature was 102°; on 9. vi, at noon, it was 100°, in the afternoon, 102°; on 10. vi, at noon, it was 101°, in the afternoon it was 100·8°; on 11. vi, at noon, it was 99·2°, and in the afternoon 101°. After this the temperature, taken once daily, was normal. He did not complain of any symptoms. The second injection led, therefore, to a doubtful, but probably a negative, result, as the definite febrile reaction that occurred did so fifteen days after the inoculation, suggesting that it arose from some other cause.

Case IV. *Wm*, aet. 27, male, laboratory assistant.

14. v. 16. Between 11.50 a.m. and 12.30 p.m. he was bitten by *Stegomyia*; thirty-six bites were counted.

15. v. At 12.30 p.m., about twenty-two *Stegomyia* bit; at 4.30 p.m., about fourteen *Stegomyia* bit.

Cold in the head during the last few days, but subsiding on 20. v.

20. v. On going to bed he had headache, and passed a bad night. He had a sore throat. The temperature was not taken. The onset occurred at about 9 p.m. The shortest possible incubation period was five days five hours, and the longest possible incubation period was six days nine hours.

21. v. He said that his "eyes, ears and all joints and parts of body are painful." He stopped in bed all day. The headache was frontal, at the back of the head and "behind the eyes." His gums were tender. There was pain in the neck and spine; it was very bad in the lumbar region. "Every part of body was aching." Nausea was present, and he had no appetite; there was no diarrhoea; no delirium was noted;

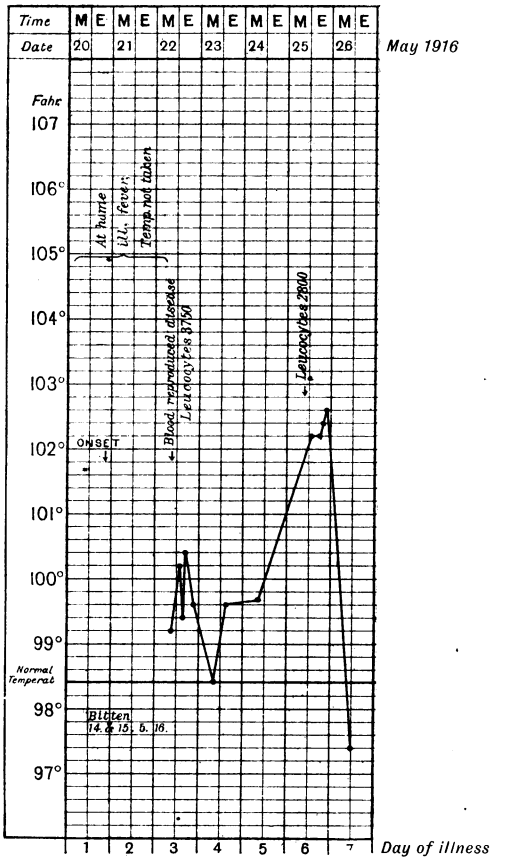


Chart III. Mosquito Case IV. Wm.

he was slightly constipated. He had no cough, but a sore throat. Some coryza was present. The temperature was not taken.

22. v. The patient got up at 8 a.m. Giddiness was present, and shivering. He vomited twice, and was much nauseated, and had no appetite. He came in to be examined. A definite rash, "midway between those of scarlet fever and measles," was found on the arms. It was doubtful on the back. This man said that his rash was often very definite on the arms on waking in the morning, but faded later. He looked ill, and shewed a swollen, typical "dengue face." The tongue was furred in

the centre and slightly strawberry at the tip. He was sent home too ill to work, and was very nauseated in the tram. The temperature on arrival at the laboratory was 99·6°, and the pulse-rate 82. The temperature later in the day was higher (see chart). Blood was taken from a vein for injection experiments. The Wassermann reaction was negative. Blood examination: leucocytes, 3700; polymorphonuclears, 53 per cent.; large mononuclears, 9 per cent.; lymphocytes, 30 per cent.; transitionals, 8 per cent. No parasites found. Red cells normal. (Amalgamated count by J. B. C. and B. B. Only 100 cells counted altogether.)

23. v. On rising, at about 9.30 a.m., the temperature was 98·4° F. On arrival at the laboratory at 10.15 a.m. he complained of an oppressive feeling in the chest and headache. There was a rash on his back, subcuticular, measly, mottled, not well marked. It was still visible on the arms, especially on the under-side of the forearms. At 4 p.m. he went home, as his back and head felt too bad to continue work, and he looked flushed and sick. His temperature was then 99·6° F.

24. v. He did not feel very well on rising, but was fairly well afterwards. His temperature, taken once only, was 99·7° F., and his pulse-rate 96.

25. v. In the morning he felt fairly well, but towards 2 p.m. felt much worse. The headache was severe and there was a tired aching in his limbs. The temperature was 102·2°. Blood examination: leucocytes, 2800; polymorphonuclears, 59 per cent.; lymphocytes, 30 per cent.; mononuclears, 7·5 per cent.; transitionals, 7·5 per cent.; eosinophiles, 1 per cent. (Two hundred cells counted.) Red cells normal. No parasites. The Widal reaction was negative. Rash. This was definite, but slight, on the back, chest and abdomen, and on the anterior internal surface of the right upper arm over the biceps near the rolled up cuffs, was a collection of slightly raised papules, which disappeared in a day or so. Elsewhere there was a faint measly rash.

26. v. The temperature was subnormal. The man returned to his work nearly well. After this he regarded himself as well.

Blood from this case, taken on 22. v. 16, was injected into volunteer P. S. (Case 29) on 24. v. The latter complained of drowsiness and aching eyes at 1 p.m. on 29. v, but his temperature was subnormal, and he was placed in bed at 6 p.m. on 30. v, when his temperature was found to be 101° F. He went through a typical attack of dengue, with rash and slow pulse, but without the double temperature curve.

Case V. *M.*, 27, female, trained nurse. Previous history: She lived in the North Coast district about eight years ago. About eight or nine years ago she had two attacks of (?) dengue (a year or more apart). She spoke of the sudden onset and extreme pains, but says she did not notice a rash.

16. v. 16. She was bitten by eighteen *Stegomyia* at noon.

25. v. She was well all day, until about 10 p.m. While sitting sewing and listening to music, she suddenly felt sick and tired, with pains in her knees, and went to bed shivering, and did not sleep until 4 a.m. She said she did not feel as if feverish, and did not take her temperature. Incubation period: nine days and ten hours.

26. v. She got up with a headache over the eyes and across the temples. The eyes were painful to move. The morning temperature was 98·6° F. She worked

all day, though not feeling well, having some nausea, but no vomiting. Occasional shivering occurred. The tongue was clean. Her eyes were slightly red and the conjunctivae of the lids swollen; no coryza or cough was noted. She complained of slight sore throat. The fauces were slightly red, but nothing very definite was seen. Temperature: 6.15 p.m., 99·6°; 9 p.m., 100·6°. She stated that she had no rash.

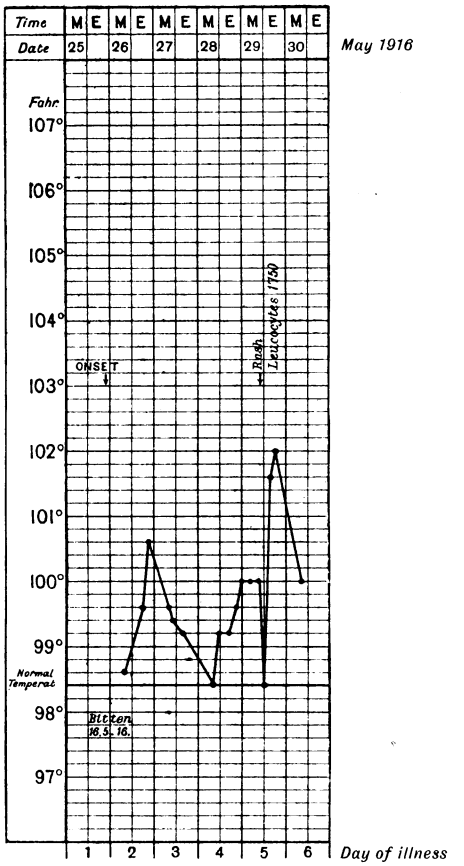


Chart IV. Mosquito Case V. M.

27. v. The temperature at 7 a.m. was 99·6° F.; at 11 a.m. it was 99·4°, and at 4 p.m. 99·2°. The eyes were slightly jaundiced, and the ears slightly yellow, but she said the jaundice was more marked before she got ill on 25. v. 16. The face was flushed, and she said her eyes were painful on movement, but the other symptoms were better. She had no pains in the neck, but had pains across the back and down the back of the legs, and occasionally a feeling of nausea. No rash was noted. She was not examined, except her arms, face, etc.

28. v. She stopped in bed, as she usually did on Sunday morning, for rest. On examination (B. B.) the temperature was normal. The skin was mottled over the back, chest and arms, not a definite rash, but abnormal. The elbows were red and pimply-looking, not very marked. She said she felt weak. Glands were found enlarged in the anterior triangle of the neck, on the left side; but these may have been present before. The temperature went up in the evening (see chart).

29. v. At 9 a.m. she stated that she had had a very bad night, and could not rest at all. She had a recurrence of symptoms, and felt and looked sick. The headache was severe last night, and she took aspirin with relief. On rising, the Matron described a well-marked, measly rash on her arms, which faded on exposure to cold. It was scarcely perceptible at 9 a.m. A mottled, indistinct rash was now on the back. The elbows shewed a very marked and curious condition. The affected areas were about the size of a crown-piece, red, raised, of a bright pink colour, and in the outlying parts were separate papules. She said they were painful to touch. This most distinct condition was seen by the Matron and B. B. Blood examination: no parasites seen. Red cells normal. Leucocytes (duplicate counts made), nineteen whole millimetre fields counted, 1750. The morning temperature was 100·2°; at noon it was normal, and it was up again at night to 102°. The tongue was furred in the centre, and strawberry-like at the tip.

30. v. The patient said she had had a very bad night. Yesterday afternoon she got worse, felt very ill and could not rest at all at night. She could not lie in any position. She said never before had she felt so bad. On examination at 9 a.m., she looked ill; her face was flushed. The tongue was dirty, but not as much as yesterday. The temperature was 99·8°.

The rash was very marked on both arms. It was for the most part of a dark purplish pink colour, and measly in type. It was most marked on the external and extensor surfaces of the fore and upper arms to the shoulders. Over the elbows it was more raised and slightly papular. Very distinct discrete macules were fairly numerous on the palms of the hands. On the upper chest and on the upper back there was an indefinite mottling; on the lower back the mottling was more marked, but not as distinct as on previous occasions. On the knees there was a discrete, small papular rash over the anterior surface for about six to eight inches. On the ankles there was a similar discrete, papular rash on the anterior surface, extending about four inches up the leg and slightly on to the dorsum of the foot.

She was seen by Drs Paton, Armstrong, Van Someren, Woolnough, and by Dr Bligh on the evening before, when the rash was distinct.

She stated to one of us later that, about this time, the rash was marked on the abdomen.

After 30. v. we did not see this patient, and she stopped taking her temperature, but she informs us that she was feeling sick at irregular intervals for a day or two, and suffered for about a week from marked pruritus, especially of the palms of the hands, severe enough to prevent her sleeping.

B. B. saw her next during his own illness, on Sunday, 4. vi. 16, when she was apparently quite well, but still complaining of the itching. Later she said she had occasional headaches for about a week after 30. v.

Case VI. *B. B.*, aet. 34, male, medical practitioner.

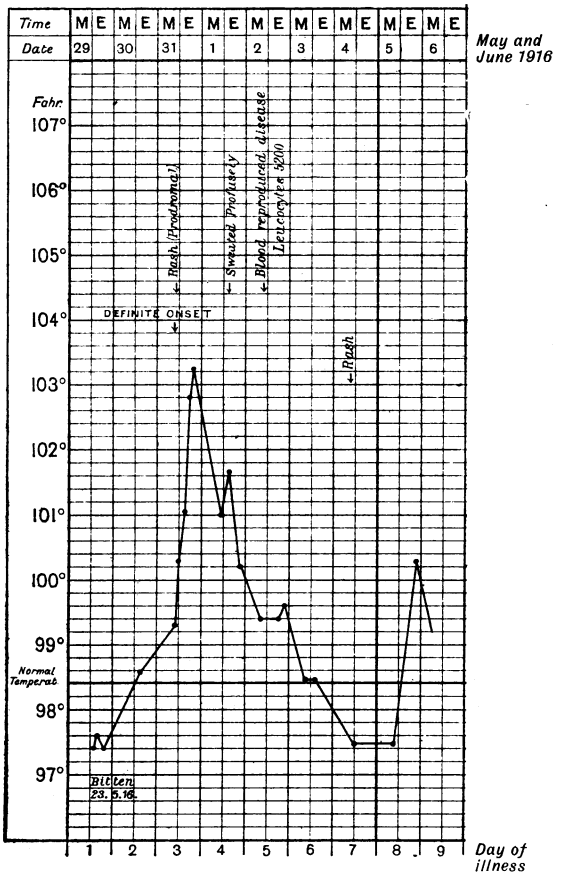


Chart V. Mosquito Case VI. B. B.

13. v. 16. This was the last time B. B. was in the dengue natural area. He was bitten by mixed Grafton mosquitoes.

14. v. He was bitten by mixed Grafton mosquitoes, and arrived back in Sydney (mid-day).

23. v. He was bitten by *Stegomyia*. Fifteen bites were counted. Time, early afternoon.

29. v. He felt quite well on rising, but during the morning, while working, he had shooting pains in the head. In the middle of the day he had definite slight headache and a slight "tired" feeling, and slight pains in the legs and arms. The temperature at 2 p.m. was 97·4° F.; at 4 p.m., 97·6°; and at 7 p.m., 97·4°. The symptoms were so slight that B. B. felt inclined to put them down to imagination, the wish to acquire dengue being father to the thought. He now regards these symptoms as prodromal, and think they might not have been noticed in a non-expectant individual.

30. v. He was feeling "off colour," with occasional slight attacks of nausea, and had a tired sensation in the limbs and slight headache; he was worse towards evening, when he felt cold, shivery and tired, and went to bed early. He passed a rather disturbed night. The temperature at 4 p.m. was 98·4° F.

31. v. On rising, he had malaise, headache, nausea, shivering, pains all over (arms, legs, across shoulders, neck, spine, knees, ankles), and general headache. This increased during the day. The eyes were not very bad, but he was conscious of them feeling abnormal. He had slight sore throat and post-nasopharyngeal irritation, but no coryza. He felt unutterably weary, and could not concentrate his attention. The temperature at 11 a.m. was 99·3° F.; at 1 p.m. it was 100·3°, and the pulse-rate was 80; at 3.45 p.m. the temperature was 101·1°, and the pulse-rate 96. A prodromal rash was present. The incubation period was about seven and three-quarter days to the onset of fever.

Description of prodromal rash seen by Dr Chapple: "A rash resembling subcuticular petechial areas, varying in size; it is most marked on the abdomen, but also present on the back. It is most prevalent in lumbar region posteriorly. The arms are not markedly affected. Each area shews no point of deepest intensity, and the edge is not sharply defined. The colour does not completely disappear on pressure. The colour might be described as a light raw ham colour, with a faint tinge of purple."

J. B. C. Obscure mottling on the trunk; on the back the hair follicles prominent.

B. B. was seen by several medical men. All agree that a distinct rash was present.

Later in the afternoon he felt worse, but managed to work until 4.30, when he went home. He arrived home at 6 p.m. At 6.30 p.m. the temperature was 102·8° F.; at 8 p.m. it was 103·3°, and the pulse-rate was 116.

Note at 8 p.m. The patient was sitting by a gas fire; he felt very hot, but not very ill. Body pains, while resting, were not troublesome. He was very nauseated after tea, of which he ate moderately.

After going to bed, at 9 p.m., he had slight vomiting and marked nausea, and was very restless in bed, the pains in the ankles being maddening. He could only rest by protruding his feet outside the clothes, and could not bear the weight of the clothes. Once he got to sleep he slept well.

1. vi. 16. The patient lay in bed all day. The headache was bad, and was accompanied by photophobia and eye pain. Shivering occurred at times, and giddiness on standing. He sweated twice profusely late in the day. There was slight mental wandering at night, but he slept fairly well.

2. vi. He woke up with headache and eye pains still present, but feeling better, and went into town, though feeling rather shaky, very tired and depressed, nauseated and headachy. The temperature in the morning was 99·0°, leucocytes, 5200. Blood drawn for injection experiments. He went to bed at 7.30 p.m.

A person (G. D., Case 26–32) inoculated with blood taken on 2. vi. 16, developed typical mild dengue. Another person (E. H. R., Case 30), subject of a previous experimental attack, remained well (see injection results).

3. vi. He did nothing all day. Headache was present, and his eyes were tender. He felt very depressed.

4. vi. The patient felt much the same as on the preceding day. The nausea was marked, especially after food. There was a well-marked rash all over the trunk. This rash was distinctly different in colour from the prodromal rash. The lesion

might be described as an irregular, fairly bright mottling of the skin; irregular dark areas and irregular pale areas alternated. On the darker areas were brighter punctiform lesions. One of us (B. B.) likens the rash to the strawberry. The rash was best seen early in the morning, and was then noticed on the back of the wrists and on the flanks and back, being less marked on the abdomen. Later in the course of the illness it was well-marked on the forearms. The elbows were somewhat red, but not distinctly so; the flexor and extensor surfaces were both involved; the rash was most apparent on the flexor surfaces of the forearms, and was slight on the external aspect of the buttocks and the anterior aspect of the knees, and doubtful on the ankles and palms. The rash was seen by Drs Cleland, W. G. Armstrong, Paton, Woolnough, Isbister, Chapple and others, who all concur as to its definite characters.

5. vi. He felt worse than on the previous day. He was intensely cold, and shivered immoderately. The rash was well-marked on the arms, wrists and trunk. The temperature was subnormal. Nausea and attacks of great giddiness occurred. In the evening, the temperature rose to 100·3°.

6. vi. He awakened feeling better, and apparently afebrile, and had a good breakfast. He then began work. The temperature at mid-day was 98·1°. The rash was still well-marked; it was seen by Drs Cleland, Paton and Armstrong.

7. vi. He still had headache and slight tenderness on moving his eyes. There was still some nausea and tiredness. The temperature at 11 a.m. was 97·6°.

For the rest of the week he was not feeling "himself," although afebrile. There was a tendency to have headache during part of the day, and pain on moving the eyes and stiffness in the joints, back, etc. A bad taste in the mouth was noted, and inability to enjoy smoking. There was also noted some itchiness of the skin and palms of the hands.

13. v. 16. Blood was drawn for injection experiments.

27. v. 16. The skin was peeling on the legs and hands. The patient had symmetrical, bright-coloured patches of rash on the hips and across the back, which were first noticed about a week before. There were irregular bright red areas alternating with pale skin. The skin was very irritable all over. He still had a stiff, painful feeling on rising in the morning. Otherwise he felt quite well.

The patches of rash on the hips gradually faded, leaving some staining.

INJECTION EXPERIMENTS.

G. D. (Case 26–32) swallowed blood from a previous (blood inoculation) case on 24. v. 16, and complained of pains in the head and dizziness from May 28th (four days later) to May 31st, but had no rise of temperature, the temperature being taken once daily. On 2. vi. 16, he was given 1 c.c. of blood from B. B. subcutaneously. He became ill on 11. vi (eight and a half days later), and his temperature rose on 12. vi, in the evening. He had a definite attack of dengue, with a single temperature curve.

Another experiment was made with the same specimen of blood; 1 c.c. was injected into a volunteer (E. H. R., Case 30), who had passed through a typical attack of experimental dengue, commencing on 25. v. 16, and terminating on 30. v (Case 13). No symptoms followed this second injection within a period of fourteen days.

With a specimen of blood taken on 13. vi. 16 (fourteen days from the onset of B. B.'s illness), a volunteer (G. R., Case 31) was injected with 8 minims. No symptoms or signs of dengue followed during the subsequent nine days.

APPENDIX IV

TABULATED STATEMENT OF THE DETAILS OF THE INOCULATIONS AND ALLIED EXPERIMENTS.

No.	Initials and age	Material used for experiment: Injection 1st	Material used for experiment: Injection 2nd	Day of illness on which material was taken: Injection 1st	Day of illness on which material was taken: Injection 2nd	Date of collection of material 1st	Date of collection of material 2nd	Date of injection: Injection 1st	Date of injection: Injection 2nd	Period material was outside body: Injection 1st	Period material was outside body: Injection 2nd	Result	Bordet (Wassermann)	Incubation period
1	J. H., 40	4 A	—	3rd	—	5. iv. 16		8. iv. 16	—	3 days	—	Neg.	—	—
2	G. C., 43	4 A	XX	3rd	8th	5. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	3 days	½ day	Neg.	—	—
3	R. W., 43	4 A	XX	3rd	8th	5. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	3 days	½ day	?	—	30. iv. 16 22 or 18 days
4	M. J. W., 46	4 A	XX	3rd	8th	5. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	3 days	½ day	Pos.	—	19. iv. 16 11 or 7 days
5	J. B., 48	4 A	XX	3rd	8th	5. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	3 days	½ day	Neg.	—	—
6	W. McG., 48	1 C	XZ	3rd	8th	4. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	4 days	½ day	Pos.	Neg.	16. iv. 16 8 or 4 days
7	J. C., 49	1 C	XX	3rd	8th	4. iv. 16 12. iv. 16		8. iv. 16	12. iv. 16	4 days	½ day	Pos.	Pos.	16. iv. 16 8 or 4 days
8	J. McA., 53	1 B	XX	3rd	8th	4. iv. 16 10. iv. 16		8. iv. 16	12. iv. 16	4 days	½ day	Neg.	—	—
9	J. E., 56	1 B	XZ	3rd	8th	4. iv. 16 10. iv. 16		8. iv. 16	12. iv. 16	4 days	½ day	Pos.	—	19. iv. 16 11 or 7 days
10	J. D., 64	YA Corpuscles from Case E		3rd		14. iv. 16		14. iv. 16		Less than 12 hrs		Neg.	—	—
11	E. C., 51	YB Serum from Case E		3rd		14. iv. 16		14. iv. 16		Less than 12 hrs		Pos.	Neg.	23. iv. 16 8½ days
12	G. J., 47	Filtered blood from Case 6		2nd		17. iv. 16		18. iv. 16		1 day		Pos.	Neg.	25. iv. 16 6½ days
13	E. H., 44	Blood, Case 6		2nd		17. iv. 16		18. iv. 16		1 day		Pos.	Neg.	25. iv. 16 6½ days

14	T. H., 59	Corpuscles, Case 11	4th	26. iv. 16	27. iv. 16	1 day	Neg.	—	—
15	E. C., 45	Washings, Case 11	4th	26. iv. 16	27. iv. 16	1 day	Neg.	—	—
16	G. R., 55	Corpuscles, Case 13	2nd	26. iv. 16	27. iv. 16	1 day	Pos.	Neg.	3. v. 16 6 days
17	W. W., 38	Washings, Case 13	2nd	26. iv. 16	27. iv. 16	1 day	Pos.	Neg.	? 3. v. 16 6–7 days
18	J. T., 64	Filtered blood from Case D	6th	21. iv. 16	28. iv. 16	7 days	Neg.	—	—
19	McS., 65	Filtrate from Case 11	4th	26. iv. 16	28. iv. 16	2 days	Neg.	—	—
20	J. P., 56	Filtrate from Case 12	2nd	26. iv. 16	4. v. 16	8 days	Neg.	—	—
21	W. F., 63	Filtrate from Case 13	2nd	26. iv. 16	4. v. 16	8 days	Neg. ?	—	—
22	A. C., 19	*Vaccinated* with serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Pos. ?	Neg.	—
23	R. K., 48	*Nostrils swabbed* with serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Neg. ?	Neg.	—
24	W. C., 30	*Swallowed* serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Pos. ?	—	—
25	L. J., 52	Serum from No. 17	3rd	5. v. 16	12. v. 16	7 days	Pos.	Neg.	21. v. 16 9 days
26	G. D., 67	*Swallowed* serum from 25	? 4th	? 24. v. 16	24. v. 16	? 4 days	Neg. ?	—	—
27	H. K., 50	Blood from J. G., No. 1	2nd	20. v. 16	24. v. 16	4 days	Pos.	—	1. vi. 16 8 days
28	N. M., 63	Blood from J. G., No. 2	4th	22. v. 16	24. v. 16	2 days	Neg. ?	—	—
29	P. S., 46	Blood from Wm.	3rd	22. v. 16	24. v. 16	2 days	Pos.	—	? 30. v. 16 5–6 days
30.	E. H., 44	Blood from B. B., No. 1	3rd	2. vi. 16	2. vi. 16	3 hours	Neg.	—	—
31	G. R., 49	Blood from B. B., No. 2	14th	13. vi. 16	13. vi. 16	3 hours	Neg.	—	—
32	G. D., 67	Blood from B. B., No. 1	3rd	2. vi. 16	2. vi. 16	3 hours	Pos.	—	11. vi. 16 8½ days

APPENDIX V.

HISTORIES OF CASES IN WHICH MATERIALS FROM CASES OF DENGUE WERE INJECTED, ETC.

Subcutaneous Injection of Filtrate of Citrated Blood taken on the third day of the Natural Disease (Case A), outside the body three days. *Result*: negative.

Case 1. *J. H.*, m., 48, was injected subcutaneously 8. iv. 16 at 3 p.m., with 1 c.c. of filtrate 4 A (Pasteur-Chamberland filtrate of citrated blood of Natural Case A taken on the third day of the disease on 5. iv. 16). The patient remained well, being under observation for at least twelve days. The result was negative.

Same injection as Case 1, followed four days later by a Subcutaneous Injection of whole Citrated Blood taken on the eighth day of the Natural Disease (Case C), outside the body half-day. *Result*: negative.

Case 2. *G. C., m.*, 43, was injected subcutaneously on 8. iv. 16 at 3 p.m., with 1 c.c. of filtrate 4 A (see above), and on 12. iv. was again injected subcutaneously at 7.30 p.m. with 1 c.c. XX (whole citrated blood from Natural Case C taken on the eighth day of the disease, 12. iv.). This case remained well, being under observation at least eighteen days.

The same injections as Case 2, save that the second one was Intramuscular. *Result*: an illness of a doubtful nature beginning twenty-two days after the first injection and eighteen days after the second.

Case 3. *R. W., m.*, 43, was injected subcutaneously with 1 c.c. of filtrate 4 A (see Case 1), on 8. iv. 16, at 3 p.m., and with 1 c.c. XX intramuscularly on 12. iv, at 7.30 p.m. He became suddenly ill eighteen days later on 30. iv, about mid-day, complaining of headache, aching eyes and a feeling of drowsiness. He was placed in hospital and on examination on 1. v. shewed a flushed face with injected eyes, a furred tongue, and an injected pharynx, presenting an appearance suggestive of a mild attack of dengue. His skin was hot and a faint rash seemed beginning to appear on the back. The back shewed an erythematous condition and was very sensitive to pressure. This flushed condition of the back was constant. When questioned regarding any running from the nose, he stated he was suffering in that way but it was not apparent at this or at subsequent daily examinations. The morning temperature was 98·6° F., and the evening one 98·4°.

2. v. 16. The headache was still present and the eyes aching and heavy. There was a pink mottling confined to the back with the congested condition of the back still present. Temperature, morning, subnormal; midday, 99°; evening, subnormal.

3. v. No symptoms. The rash extended round the flanks but was not seen in any other area. Temperature, subnormal.

4. v. No symptoms. The rash was fainter but could be demonstrated on the back and less distinctly on the abdomen. Temperature, subnormal.

6. v. No symptoms. The rash was faintly distinguishable on the back. Temperature, subnormal.

7. v. No symptoms. Still a faint pink mottling on the back. Tongue still coated. Temperature, subnormal.

8. v. No symptoms. The rash seemed to be more demonstrable and to be present on the back, abdomen and lower part of the chest. Urine, 1015, acid, no albumen or sugar. Temperature normal.

9. v. No symptoms. The rash has the same distribution as on the previous day. Temperature subnormal.

10. v. ditto.

12. v. No symptoms. The patient was allowed up, the pink mottling of the back being still present.

(During the period 2. v–12. v. 16 he sweated very freely at night—nurse's report.)

19. v. Re-examined. No obvious rash. Feels well but complains of weakness. Temperature subnormal.

20. v. Discharged from hospital. Temperature subnormal.

2. vi. He became ill again with "headache and aches all over."

3. vi. He was sent into hospital. Evening temperature 101°.

4. vi. On examination his face was found to be flushed; the eyes were injected and watery; there was no running from the nose; the tongue was thickly coated; there was a definite congested condition of the back but no rash. He states he "feels very ill with a terrible headache." Temperature, morning, 101·6°; evening, 101°.

5. vi. Headache still bad; tongue coated; no rash. Temperature, morning, 98·6°; evening, 98·8°.

6. vi. Seems well but still complains of headache; eyes not watery; tongue clean. Temperature, morning, 98·4°; evening, 98·6°.

7. vi. Complained of profuse sweating at night since admission; slight headache and occasional cough; tongue cleaning. Temperature, morning, normal; noon, 99°; evening, 98·4°.

8. vi. Pains in the back (lumbar region); no rash; no sweating. Temperature after this date normal or subnormal.

9. vi. Had a good night; feels well; no rash.

10. vi. Feels well. Urine, 1030, acid, no albumen, reduction of Fehling's solution.

11. vi. Still feeling well. Urine, 1026, no albumen, acid, reduction of Fehling's solution.

12. vi. Well. Urine, 1006, acid, no albumen, no reduction of Fehling's solution.

15. vi. Well. Discharged.

Although the symptoms were suggestive and some rash was present, the length of the incubation period, and absence of fever in the first attack make it seem probable that this case was not one of dengue. In any case it is impossible to include it in our positive results.

The same Injections as Case 3. *Result*: positive, not marked. *Incubation period*: seven days from second injection, or eleven days from first injection.

Case 4. *M. J. W., m.*, 46, was injected subcutaneously with 1 c.c. 4 A (see above) on 8. iv. 16 at 3 p.m., and on 12. iv. at 7.30 p.m. intramuscularly with 1 c.c. of XX (see above).

He stated he had never felt perfectly well since the second injection but became definitely sick on the night of 19. iv. with "a feverish feeling and darting pains like rheumatism in all his joints particularly the knees and shoulders." He also had occipital headache.

20. iv. On examination there was found to be flushing of the face; slight injection of the eyes; no coryza; a coated moist tongue; no rash, but an erythematous condition of the back. He complained of pains all over the body, particularly in the joints. Temperature, morning, 101°; evening, 99·8° F.

21. iv. Feels well; no rash. Temperature, morning, normal; noon, 99·8°; evening, 99·8°.

22. iv. Feels well; no rash. Temperature, morning, 99·4°; evening, 99·4°. (Drop to 97·4° at 4 p.m.)

23. iv. The face seems more flushed. Complains of headache, and pains in the right shoulder. Temperature, morning, subnormal; afternoon, 99·8°.

24. iv. Pains in the legs and shoulders, with headache. Temperature, morning, 99·8°; evening, 100°.

25. iv. Slight headache with pains in the muscles of the lower half of the body; tongue still coated and moist. Temperature, morning, subnormal; evening, 100·2°.

26. iv. "Rheumatic pains in the knees and hips, and in the muscles from the hips down"; complains of sleeping badly. No rash. Temperature, morning, subnormal; evening, 99·6°.

27. iv. Occasional pains in the knees and shoulder joint; no rash. Temperature, morning, subnormal; evening, 99°.

28. iv. Still pains in the knees; feels well. Urine clear, 1018, acid, no albumen or sugar. Temperature, morning, subnormal; evening, 99°.

29. iv. Pains in the elbows and shoulders. Temperature, morning, subnormal; evening, 99°.

30. iv. Pains in the knees; headache. Temperature, morning, subnormal; evening, 99°.

1. v. Pains in the knees and hips. Temperature, morning, subnormal; evening 99°.

2–5. v. Pains in the knees and hips. Temperature, morning, subnormal; evening temperature on 2. v. 16, 99°—after this not above normal.

6–12. v. Feels well. Temperature subnormal. Discharged on latter date.

Urine (undated), 1020, acid, no albumen or sugar.

Remarks.

A review of the chart shews some approximation to the double type. This is undoubtedly an irregular diphasic variation with the high points at noon an April 20th and 8 p.m. on April 25th. There are however several intermissions. Relative bradycardia is very marked after the first day, and periods of absolute bradycardia are frequent. The temperature took a considerable period to settle down as seen by reference to the chart.

This was the only case in which the patient described the pains as being "rheumatic." There was no tenderness in or around any of the joints of which complaint was made, so that acute rheumatism could be excluded.

The case is considered a positive one with the invasion on the night of 19. iv. 16,

thus giving an incubation period of seven days from the second injection or eleven days from the first injection. The case was under observation for thirty-five days from the first injection.

Same Injections as Case 3. *Result*: negative.

Case 5. *J. B., m.*, 48, was injected *subcutaneously* on 8. iv. 16 with 1 c.c. of 4 A (see above), and *intramuscularly* on 12. iv. with 1 c.c. of XX (see above).

This case remained well, being under observation at least twelve days. The result was negative.

Subcutaneous Injection of Serum and Corpuscles, taken on the third day of the Natural Disease (Case B) outside the body four days, followed four days later by a Subcutaneous Injection of Serum, taken on the eighth day of the Natural Disease (Case C), outside the body half-day. *Result*: positive. *Incubation Period*: eight days from the first injection, or four days from the second injection.

Case 6. *Wm. McG., m.*, 48, was injected with 0·5 c.c. of 1 C (serum and corpuscles from Case B) on 8. iv. 16 at 3 p.m. and with 0·5 c.c. of XZ (serum from Case C) at 7.30 p.m. on 12. iv. He became suddenly ill on the night of 16. iv. with frontal headache, a "feeling of cold and hot all over," and a dead aching pain in the legs and lumbar region and an acute sharp pain in the back of the neck.

17. iv. 16. On examination, face flushed, particularly the forehead; eyes slightly injected; injection of pharynx; tongue coated and moist. Appetite good. No rash. Sweated freely during the night; slept well. Temperature at 10.30 a.m., 102·3° F.; noon, 100°; midnight, 102°.

The Pasteur-Chamberland filtrate of clot and serum, and the untreated serum and corpuscles obtained from the blood of this case on this date, conveyed the disease to Cases 12 and 13 respectively, after an incubation period in each case of six and a half days.

18. iv. Slight headache; pains nearly gone; feels fairly well; skin active; pains in the muscles of the arms. *Rash.*—A scarlet flush in the axillary line round the waist and on the buttocks (pressure?). Temperature, 8 a.m., 99°; noon, normal; 8 p.m., 100·6°.

19. iv. Face less flushed; tongue cleaning; feeling fairly well; a faint blotchy erythematous rash on the back and shoulders. Temperature, 8 a.m., 98·6°; noon, 100·2°.

20–21. iv. Feels well; rash the same. Highest temperature, 99·4°.

22. iv. Feels well. In the evening of this day (midnight), the temperature rose to 102·6°.

23. iv. Feels well. Temperature, 8 a.m.. 100·6°; 8 p.m., 102·2°.

24. iv. Eyes aching; otherwise well. Temperature, 8 a.m., 99°; 8 p.m., 99·8°.

25–27. iv. Feels well; rash the same. The highest temperature was 99·4°.

28. iv. Feels well; rash the same; allowed up. Urine clear, 1025, acid, no albumen or sugar. Temperature normal.

3. v. Feels fairly well.

4–10. v. Quite well but "weak in the legs."

The urine tested on two other occasions shewed:

A. 1010, acid, no albumen or sugar.

B. 1030, acid, no albumen or sugar.

Remarks.

The incubation period was approximately eight days from the first injection, or approximately four days from the second injection.

The duration of illness was about twelve days. The patient was under observation thirty-two days.

The chart shews definitely a double stage of pyrexia, and is the most typical saddle-back chart in our series of injection cases. In this connection one cannot overlook the double injection and the possibility of the double temperature phase being related to this, but consideration of several other cases, notably Case 25, following a single injection, and consideration of certain of the mosquito cases, does not lend support to such a hypothesis. The saddle-back temperature in this case is a classical feature seen in a moderate number of the natural cases, and probably depends on causes not yet understood. The lowest pulse-rate observed was 54 (3. v. 16). The pulse curve follows fairly closely the first access of pyrexia, although it is relatively somewhat slow. During the second rise of temperature the pulse curve remains on approximately the normal level, indicating a definite relative bradycardia. Absolute bradycardia is not marked in this case, although on one occasion the pulse was 54. Although the temperature was moderately high and the case definitely positive (see subsequent inoculations—Cases 12 and 13), the patient's general condition was very good and he made practically no complaint. In fact he complained more after the disease than during it, stating he had "gone off his legs." The rash was very faint.

Subcutaneous injection of Serum and Corpuscles, taken on the third day of the Natural Disease (Case B) and outside the body for four days, followed four days later by the Subcutaneous Injection of citrated blood taken on the eighth day of the Natural Disease (Case C). (Outside body half-day.) *Result*: positive. *Incubation period*: eight days from the first injection and four days from the second injection.

Case 7. *J. C., m.*, 49, who was injected subcutaneously with 0·5 c.c. of 1 C (untreated serum and corpuscles from Case B) on 8. iv. 16 at 3 p.m., and with XX (citrated blood from Case C) on 12. iv. 16 at 7.30 p.m., became suddenly ill on 16. iv. 16 at 11 a.m. with occipital headache and "shivery feelings."

17. iv. 16. On examination: Face flushed; no injection of eyes; no coryza; pharynx injected; tongue moist and coated; headache in all regions. *Rash.*—A pinkish, definitely raised, erythematous rash confined to the inner sides of both thighs. Temperature: noon, 99·4° F.; rose to 101·4° at 4 p.m.; at midnight, 99·6°.

18. iv. Headache; feels better; forehead, face and neck flushed like sunburn. Slight pinkish erythematous patches on the front of the chest; large patches in the axillary line and round the waist; rash copious on the back, over the glutei and on the back of the thighs and on their front and inner aspects; slight on the legs; also present on the palmar aspects of the forearms and slightly on the upper arm. Temperature, 8 a.m., 99°; noon, 98·4°; midnight, 99·8°.

19. iv. Still complains of pain in the back of the neck and headache. A much more prominent, bright pink, definitely raised, erythematous symmetrical rash on the inner and front aspects of the thighs; one similar patch on the back. Highest temperature at 4 p.m., 99°.

20. iv. Feels well. Copious pinkish raised patches on both thighs and both forearms; an erythematous blush on the right arm in the morning, and on the same region in the afternoon a definitely *raised* erythematous rash; the same appearance in the lumbar region; rash very itchy. Highest temperature, midnight, 99·8°; other times normal.

21. iv. Feels well. Rash covering most of the body but particularly on the lumbar and gluteal regions; faint on the forearms and thighs. Highest temperatures, noon, 99°; midnight, 99°.

22. iv. Feels well; rash fading. Temperature normal.

23. iv. Feels well; rash almost gone. Temperature normal.

25. iv. Feels well. Temperature, 4 p.m., 99°; other times normal.

26. iv. Allowed up. Temperature normal on and after this date.

28. iv. Urine clear, 1020, no albumen or sugar.

3. v. Complains of pain in the knees. Fine desquamation present.

8. v. Urine 1020, acid, no albumen or sugar.

Remarks.

This case was under observation for thirty-five days from the first injection. The incubation period was eight days calculated from the first injection, and four days from the second injection.

The chart shews some indication of a double temperature curve, with approximately three days between the two high readings of the temperature chart. Relative bradycardia is present in the pyrexial period, followed by absolute bradycardiac periods. Several times the pulse was 56 and on one occasion 54. Later in the record periods of bradycardia alternate with periods in which the pulse was somewhat quicker than normal. The rash in this case was a fairly distinctive one. The patient complained of great weakness after being allowed up, even up to 12. v. 16 (twenty-six days from the onset).

Subcutaneous Injection of Clear Serum taken on the third day of the Natural Disease (Case B), outside the body four days, followed four days later by a second Subcutaneous Injection of Citrated Blood taken on the eighth day of the Natural Disease (Case C). Outside body half-day. *Result*: negative.

Case 8. *G. McA., m.*, 53, was injected subcutaneously on 8. iv. 16 at 3 p.m., with about ½ c.c. of 1 B (clear serum from Case B), and on 12. iv. at 7.30 p.m. with 1 c.c. of XX (Case C—see above). This man remained perfectly well, being under observation thirty-one days, and the temperature being taken once daily.

Subcutaneous Injection of Clear Serum taken on the third day of the Natural Disease (Case B), outside the body four days, followed four days later by a second Subcutaneous Injection of Serum taken on the eighth day of the Natural Disease (Case C), outside body half-day. *Result*: positive. *Incubation period*: eleven days from the first injection or seven days from the second injection.

Case 9. *J. E., m.*, 56, was injected subcutaneously with approximately ½ c.c. of 1 B (Case B—see above) on 8. iv. 16 at 3 p.m., and on 12. iv. at 7.30 p.m. with about ½ c.c. of XZ (serum from Case C). He became ill about 11 a.m. on 19. iv.

with a sensation of shivering. His temperature at 8 o'clock that evening was 99·4°. He complained of pains between the shoulders and in the nape of the neck, with dull pains in the legs, and dizziness.

20. iv. 16. On examination: The face was flushed; the pharynx injected; the eyes injected. There was a suggestion of an erythema on his back but nothing distinctive. He insisted that his trouble was influenzal and a slight coryza lent additional weight to his auto-diagnosis. He was not sent to bed but his temperature was frequently taken as shewn on the chart. Temperature, 100° F. in the morning.

21. iv. Temperature taken once, 98·8°.

22. iv. Temperature taken once, 98·6°.

23. iv. Temperature, morning, 99°; evening, 99·8°.

24. iv. The patient was on leave and went to the races. He had taken a good deal of drink.

25. iv. Re-examined on this date. His face was decidedly flushed. There was no evidence of coryza. He stated he felt a feeling as if he had drunk a little too much alcohol the day before. He had general pains, headache, and some malaise. His skin was examined, the patient meanwhile protesting that there was no rash on him. The examination, however, revealed a profuse rash. This was a pinkish-coloured erythematous mottling, morbilliform in character, of the whole body, including the soles of the feet, and most copious on the back. On both elbows at the same time, and less distinctly on both knees, were raised pink patches said by an observer to be the colour of "washed eosin stains."

This rash when first seen was considered distinctive enough to warrant having a water-colour record taken but on 26. iv. 16, the earliest time on which this could be done, it had faded so considerably that this procedure was considered useless. Temperature, morning, 100·6°.

26. iv. Temperature, 4 p.m., 100·6°; noon, 98·6°; evening, subnormal.

27–28. iv. Feels well. Rash fading. Urine clear, 1020, acid, no albumen or sugar. Temperature practically normal.

29. iv. Feels well. Rash fading.

1. v. Feels well. Rash fading.

2. v. Rash practically gone.

From 26. iv to 7. v, the temperature at times was slightly above normal.

7. v. Temperature up to 100°. No notes made.

Remarks.

The incubation period is six days sixteen hours, calculated from the second injection, or ten days twenty hours calculated from the first injection.

The chart shews a definite saddle-back temperature with approximately five days between the highest points on the temperature chart. A complete pulse record was not taken.

Two things stand out prominently in this case—the extreme mildness of the symptoms and the distinctive character of the rash. The rash observed was evidently the "secondary rash" and the symptoms thought to be influenzal were the beginning of the disease. This patient made no complaint during convalescence.

Subcutaneous Injection of Washed Corpuscles, taken on the third day of the Natural Disease (Case E), outside the body less than twelve hours. *Result*: negative.

Case 10. *J. D., m.*, 64, was injected on 14. iv. 16 at 8.30 p.m. subcutaneously with 1 c.c. of YA (corpuscles from Natural Case E, taken on the morning of the same day, washed free from serum and citrate and suspended in normal saline). He remained well, being under observation for thirty-three days. His temperature was taken once daily.

Subcutaneous Injection of Serum, taken on the third day of the Natural Disease (Case E), outside the body less than twelve hours. *Result*: positive. *Incubation period*: eight days, thirteen hours. Unsuccessful subinoculations from blood taken on the fourth day.

Case 11. *E. C., m.*, 51, was injected 14. iv. 16 at 8.30 p.m. subcutaneously with about 1 c.c. of YB (serum of Case E), taken on the third day of the natural disease.

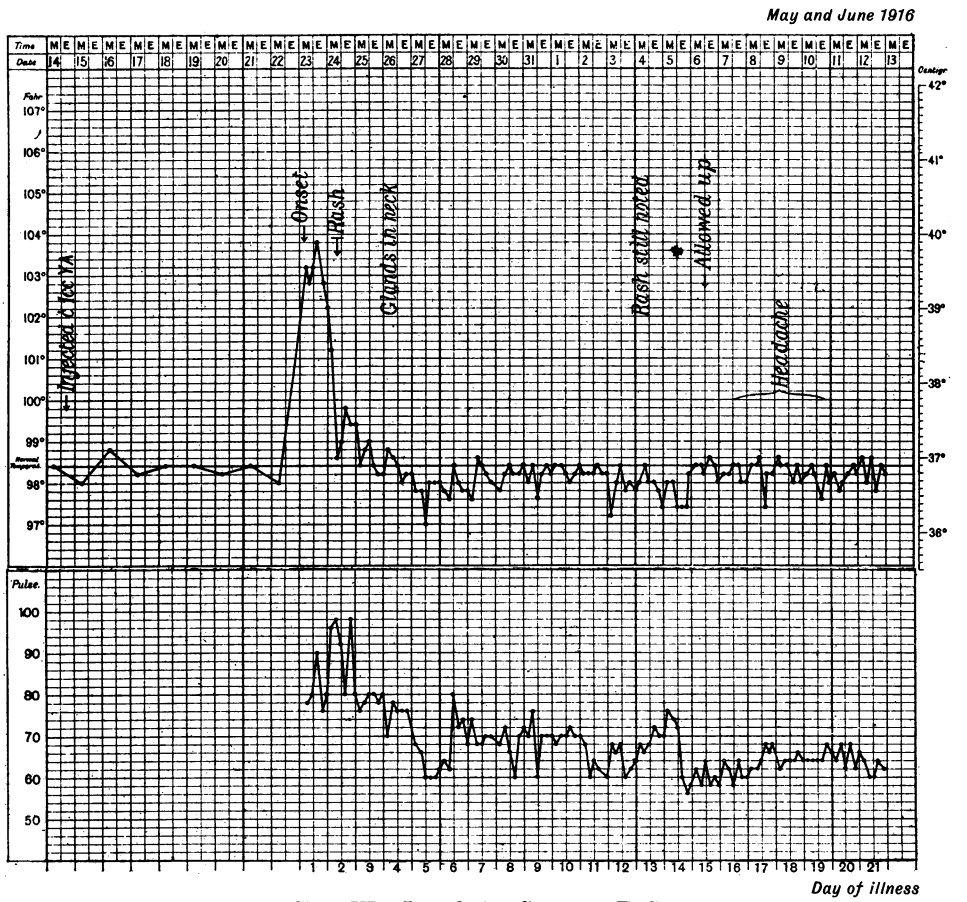


Chart VI. Inoculation Case 11. E. C.

23. iv. 16. He became ill on this day with a temperature at 9.30 a.m. of 103·1°. He complained of frontal headache and of a feeling of "being hot all over." On examination: very flushed face; no coryza; no pains; tongue moist and tremulous; pharynx injected; no rash. Temperature, 8 p.m., 103·8° F.; midnight, 102·8°.

24. iv. Headache; well-marked rash over the chest, abdomen, shoulders and thighs. Temperature, 4 a.m., 102·2°; 8 a.m., 102·2°; noon, 98·4°; 8 p.m., 99·8°.

25. iv. Headache improved. Tongue coated and moist; appetite good. A pinkish mottled erythematous rash, morbilliform in appearance, over the whole back; on the front of the chest and abdomen; on the thighs but not on the legs; and on the shoulders, arms and forearms. The rash was mostly in the upper three-fourths of the body. Temperature, 8 a.m., normal; 4 p.m., 99°; 8 p.m., normal.

26. iv. Feels well. Palpable glands in the neck. Rash fading but still prominent on the back. Temperature normal on and after this date.

The subcutaneous injection of washed corpuscles and of the citrated plasma from which the corpuscles were removed, obtained from blood taken on this date, failed to convey the disease to Cases 14 and 15 respectively. The Pasteur-Chamberland filtrate from the blood also failed to convey infection to Case 19.

The blood taken on this date and used for the above injections was treated as follows. Part was citrated and the rest allowed to clot. Some of the clear serum was tested and gave a negative Wassermann reaction. The remaining serum and clot was diluted with an equal amount of a thick emulsion of *Staphylococcus aureus* in normal saline and filtered (Pasteur-Chamberland filter). The water supply failed and in this case the first filtrate coming through was spoiled. The filter was emptied and the unfiltered material collected and refiltered next day (27. iv. 16). Cultures on broth were negative, and subcultures were negative.

The filtrate injected into Case 19 on 28. iv. 16 gave negative results.

The citrate saline mixture was centrifuged and the supernatant fluid removed and replaced with saline. Four centrifugalisations were done and the last being incomplete at 10 p.m., 26. iv. 16, the corpuscles were put into a test-tube with about 20 c.c. saline and allowed to stand in ice overnight, and in the morning were washed again twice, and the corpuscles, suspended in a small amount of saline, retained. Cultures on agar and broth and subcultures from the broth from the preparation on April 26th, and from the final preparation on 27. iv. 16, gave negative results. These corpuscles injected into Case 14 on 27. iv. 16 gave negative results. The supernatant fluid (sterile by broth and agar cultures) after the first and second centrifugalisations was used to inject Case 15 on 27. 4. 16, also with a negative result.

27. iv. Feels well; rash fading.

28. iv. Feels well; rash fading. Urine clear, 1012, acid, no albumen or sugar.

29–30. iv. Feels well; rash fading.

1–2. v. Feels well; rash fading but still noticeable on the abdomen and back.

3. v. Ditto.

4. v. Ditto.

6. v. Allowed up.

7–10. v. Complains of headache. Urine 1020, acid, no albumen or sugar.

Remarks.

The incubation period of this case was eight days thirteen hours. The temperature chart shews a very rapid rise and fall, followed by a more gradual fall to normal, but no saddle-back. The lowest pulse rate was 56. There is well-marked relative and later absolute bradycardia. The slowness of the pulse during the first part of the pyrexia is very marked. The patient complained of headache and general weakness during convalescence. He shewed a well-marked rash, morbilliform in appearance. Subinoculations unsuccessful from washed corpuscles, citrated plasma and Pasteur-Chamberland filtrate obtained from blood taken on the fourth day.

Subcutaneous Injection of Pasteur-Chamberland Filtrate of Clot and Serum, taken on the second day of the Inoculated Disease (Case 6), one day outside the body. *Result*: positive. Unsuccessful subinoculation. *Incubation period*: six days, fourteen hours.

Case 12. *G. J., m.*, 47, was injected subcutaneously on 18. iv. 16 at 7.30 p.m. with 3 c.c. of a Pasteur-Chamberland filtrate of clot and serum from Case 6. This blood was collected, 17. iv, on the second day of Case 6's illness. He became ill suddenly on 25. iv, his temperature at 9.30 a.m. being 102·2° F., and at midday 103·3°. He complained of dull headache in the occipital region and vomiting, but had no pains. On examination: Face very flushed; tongue coated; pharynx injected; slight cough; no coryza; a fine punctate rash on the back with a definite congestion of the skin; skin very hot. Temperature at 8 p.m., 103·2°; midnight, 102·2°. (See Chart VII, overleaf.)

26. iv. 16. Cough without expectoration; eyes injected; bilious vomiting early in the morning. Definite flushing of the back with a fine rash as described. Temperature, 8 a.m., 101·2°; 8 p.m., 102°.

The Pasteur-Chamberland filtrate from blood taken on this date, and injected eight days later, failed to produce the disease in Case 20.

The blood used for the above experiment was treated as follows. Part was allowed to clot and filtration was attempted with a Pasteur-Chamberland filter on the same date after dilution with an equal amount of a thick emulsion of *B. prodigiosus* in normal saline. Owing to poor and intermittent water pressure this was not achieved this day. Later, 27. iv. 16, filtration was effected and cultures from the filtrate on agar and broth and subcultures from the broth remained sterile. The filtrate was used to inject Case 20 on 4. v. 16, with negative results.

27. iv. Cough still present; slight vomiting in the morning; no pain; feels well. The fine punctiform early rash is replaced by a pink, erythematous mottling with definitely raised irregular patches on the back, chest, and abdomen and splashes of erythema on the buttocks (pressure?). Temperature, 8 a.m., 100·2°; midday, 102·2°; 8 p.m., 100·4°.

28. iv. Vomiting; cough with expectoration. Complained of slight headache. Rash the same with some blotchy erythema over the upper portion of the body. Urine clear, 1015, acid, no albumen or sugar. Temperature, 8 a.m., 99°; 8 p.m. 100·4°.

29. iv. Rash still prominent on the back and round the waist. Temperature, 8 a.m., 99·2°; midday, 100·2°; midnight, 99·4°.

30. iv. Feels well; rash still marked. Temperature, normal.

1. v. Rash seems more marked shewing copiously on the whole of the back, the buttocks, and abdomen, and slightly on the chest and upper arms. Temperature normal.

2. v. Face still very flushed; eyes red; complains of slight headache; rash still with the same pinkish mottled character and distribution. Temperature normal.

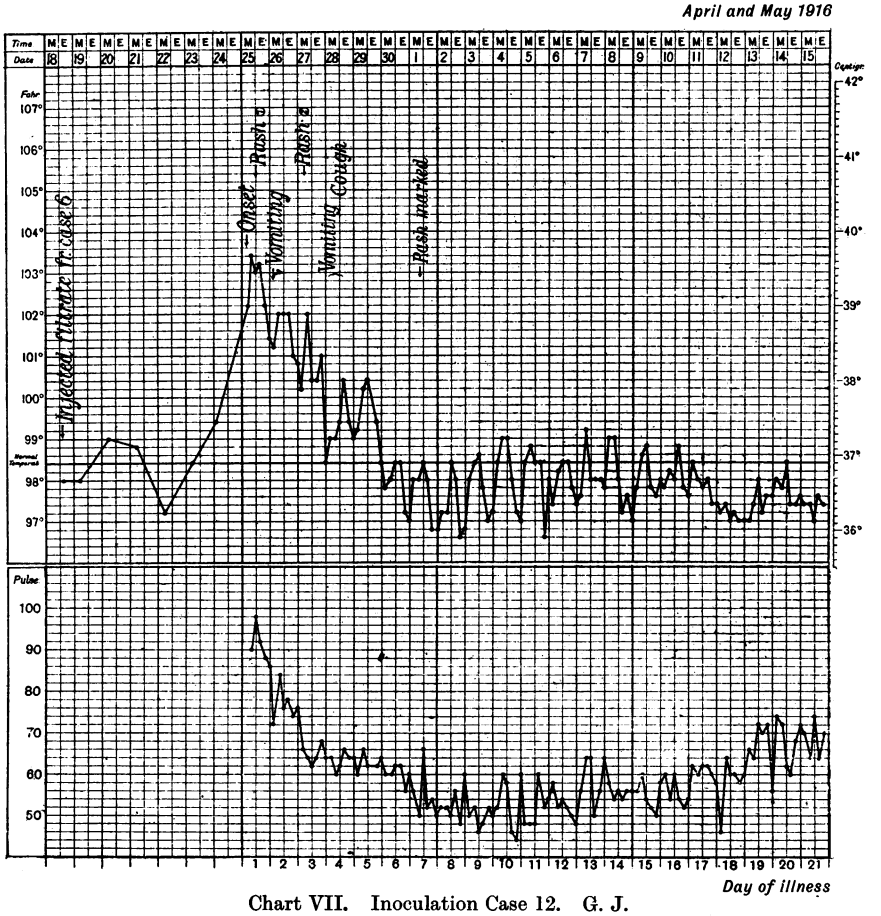


Chart VII. Inoculation Case 12. G. J.

3. v. No symptoms; rash the same. Temperature normal.

4. v. No symptoms; rash fading. Temperature 99° in the middle of the day.

5–6. v. No symptoms; rash fading.

7. v. No symptoms; rash fading.

8–13. v. No symptoms; rash fading. Discharged, 13. v. 16. Temperature, noon, 99·2°.

Urine (undated). 1025, acid, no albumen or sugar.

The case was under observation for twenty-eight days after the injection.

Remarks.

The incubation period was approximately six days, fourteen hours. The chart shews a modified saddle-back although the period of intermission was very short. There is also seen a tendency for the temperature to be above normal from the tenth to the sixteenth day after the onset. The pulse shews throughout the illness marked relative and, after the first week, absolute bradycardia. Indication of the recovery in pulse rate is found during convalescence. The lowest pulse rate was 42. The rashes were fairly prominent features. Vomiting was marked. A negative feature was no complaint of any pains at all in the body or head, other than of slight headache on the fourth day. There was a fine desquamation in this case. This patient also complained for some days of general weakness during convalescence.

Subcutaneous Injection of untreated Serum and Corpuscles, taken on the second day of the Inoculated Disease (Case 6), one day outside the body. *Result*: positive. Successful subinoculations. *Incubation period*: six days, fourteen hours (approx.).

Case 13. *E. H., m.*, 44, was injected subcutaneously with 1 c.c. of untreated serum and corpuscles from Case 6 on 18. iv. 16 at 7.30 p.m. He became suddenly ill on the morning of 25. iv. with headache, which increased in intensity during the day, and aching eyes. The patient stated he was in perfect health on 24. iv. On questioning, his headache was found to be occipital but not severe. On examination: no coryza; face flushed; hyperaemia of the pharynx; tongue furred and moist. Appetite lost.

Rash: a few punctate spots forming a faint rash on the back and between the shoulder blades; back congested. Temperature, 5 p.m., 101·6° F.; 8 p.m., 101°; midnight, 101·2°.

26. iv. 16. Complains of pains in the back all over; slept well. Eyes injected; feels well; glands palpable in the neck. Temperature, 8 a.m., 100°; midday, 102°; 8 p.m., 101°.

The Pasteur-Chamberland filtrate of blood taken on this date and kept outside the body for eight days failed to convey the disease to Case 21. The washed corpuscles after citration and also the washings from these reproduced the disease (Cases 16, 17). (Outside body one day.)

The blood used for the above experiments was treated in a similar way to that from Case 11 (*vide* this case), so that the filtrate, washed corpuscles and washings were obtained.

B. prodigiosus emulsion was used to dilute the serum and clot and filtration was successful on 26. iv. 16. Cultures from the filtrate on agar and broth and subcultures on agar from the broth remained sterile. This filtrate was injected into Case 21 with a (probably) negative result. The washed corpuscles were used to inject Case 16 on 27. iv. 16 with a positive result. Broth and agar cultures made on 26. iv. from these corpuscles were sterile at the time of injection but subsequently a slight growth appeared on the broth culture made from the final preparation. The washings (sterile by broth and agar cultures and broth subcultures), injected on 27. iv. into Case 17, gave a positive result.

27. iv. Complains of pain round the lower ribs and occipital headache. Tongue still coated. A pinkish mottling on the back and the abdomen. Temperature, 4 a.m., 101·2°; 8 a.m., normal; midday, 99°; 8 p.m., 100°.

28. iv. Slept badly during the previous night; complains of pains all over the head and round the lower ribs; feels well. Eyes still injected and tongue coated. The rash is of the same character; it is copious on the back and the abdomen; slight on the chest. Urine clear, 1022, acid, no albumen or sugar. Temperature, 4 a.m., 100°; 8 a.m., normal; midday, 99°; 8 p.m., 100°.

29. iv. Feels well. The rash is copious on the back and the abdomen, and slight on the chest. Temperature, 8 a.m., 98·6°; midday, 98·8°; 8 p.m., 98·6°.

30. iv. Feels well; appetite good. Rash still copious on the abdomen and over the whole back. Temperature normal.

1–2. v. Feels well; rash the same. Temperature normal.

3. v. Feels well; rash fading; eyes red.

4–5. v. Feels well; the rash has faded from the chest but is still present on the back and the abdomen. Temperature normal.

6–7. v. Feels well; rash fading. Temperature normal.

8. v. Feels well; the rash has faded. The patient is allowed up. Temperature normal.

9. v. Temperature, midday, 99°.

Urine (undated), 1018, acid, no albumen or sugar.

Remarks.

This case was under observation for twenty-eight days after the injection. The incubation period was approximately six days, fourteen hours. The patient states he had had "dengue" in Tasmania—a place from which the disease has never been recorded. The chart shews an irregularly remitting febrile period which may be regarded as a much modified saddle-back. There is, as in the last case, a tendency for the temperature to be above normal about a fortnight after the original onset. There is marked relative bradycardia in the latter part of the febrile stage, and occasional periods of slow pulse subsequently. The lowest pulse reading was 52. The rash was fairly copious. The patient made little complaint except during one night, that of 27. iv. 16. Washed corpuscles and also washings from corpuscles reproduced the disease in two cases. A filtrate kept eight days proved ineffective.

Subcutaneous Injection of Washed Corpuscles, taken on the fourth day of the Inoculated Disease (Case 11), outside the body one day. *Result*: negative.

Case 14. *T. H., m.*, 59, was injected subcutaneously on 27. iv. 16 at 3.45 p.m., with 1·5 c.c. of washed corpuscles (blood received into citrate normal saline solution and corpuscles washed several times in normal saline solution) taken on the fourth day of the inoculated disease (Case 11). He remained well, being under observation twenty-nine days. The temperature was taken once daily and was never over 98·4° F.

Subcutaneous Injection of washings of Citrated Plasma freed from corpuscles, taken on the fourth day of the Inoculated Disease (Case 11), outside the body one day. *Result*: negative.

Case 15. *E. C., m.*, 45, was injected subcutaneously on 27. iv. 16 at 4 p.m. with 2 c.c. of the "washings" (plasma in citrate normal saline solution after removal of the corpuscles by centrifuging) taken on the fourth day of the inoculated disease (Case 11). He remained well, being under observation twenty-four days. His temperature was taken once daily; the first day after injection it was 99° F., but thereafter it was never over 98·4°.

Subcutaneous Injection of Washed Corpuscles, taken on the second day of the inoculated disease (Case 13), outside the body one day. *Result*: positive (mild). Inoculation into this, a third individual, successful, but illness mild (perhaps due to a minimum of infective material). *Incubation period*: five days, twenty hours.

Case 16. *G. R., m.*, 55, was injected subcutaneously on 27. iv. 16 at 4.10 p.m. with 0·6 c.c. of washed corpuscles (*vide* Case 14), taken on the second day of the sub-inoculated disease (Case 13). He became suddenly ill whilst having dinner at 12.30 p.m. on 3. v. 16, with pain in the nape of the neck, headache and dizziness. His temperature that afternoon was 99° F. so he was sent into hospital for observation. On examination—his face was flushed; the tongue coated; the pharynx injected; there was slight cough; no coryza; no injection of the eyes; there was congestion of the skin of the back but no rash; there were erythematous blushes round the waist, on the thighs, and on the abdomen (pressure?).

4. v. 16. Pain in the back of the neck; no rash. Maximum temperature 98·6°, 4 p.m.

5. v. Pain in the head and neck; no rash. At midday his temperature shot suddenly up to 101°, but at 4 p.m. it was normal.

Serum obtained from blood taken on this date, mixed with similar serum from Case 17, both being kept two days outside the body, was applied by scarification of the arm, by swabbing the nostrils, and by gargling and swallowing to Cases 22, 23 and 24 respectively, with negative or very doubtful results.

6–8. v. Feels well; appetite good; slept well. No rash. Temperature normal.

9–11. v. Complained of diarrhoea and pains in the lumbar region and down the legs. No rash. On 10. v. 16 at midday the temperature was 99·2°; on 11. v. 16, the temperature at 8 a.m. was 99·6°, and at midday 99·4°.

12. v. Vomited during the preceding night. Temperature, 8 a.m. and midday, 99·6°.

13–14. v. Feels well; no diarrhoea; no rash. After this date the temperature was practically normal.

17–22. v. Feels well.

Urine (undated), 1020, acid, no albumen or sugar.

Remarks.

The incubation period was five days twenty hours. This case is considered as a mild positive one. This view is taken from the patient's appearance, symptoms, incubation period and pulse rate. The temperature chart is not typical but shews a double

phase, the two high points being separated by about five days. The pulse rate was frequently low, 56 being the lowest reading, but the pulse chart is very irregular. Additional weight is lent to the view that the case is positive by the fact that some observers considered that he had a faint rash. As however there was nothing conclusive about the rash, it was not stated in the history. Material from this case was used in three special non-inoculation experiments. Three doubtful results. (See Cases 22, 23, 24.)

Subcutaneous Injection of Citrated Plasma, taken on the second day of the Inoculated Disease (Case 13), outside the body one day. *Result*: positive. Inoculation into this, a third individual, successful. *Incubation period*: six to seven days.

Case 17. *W. J. W., m.*, 38, was injected subcutaneously on 27. iv. 16 at 4.25 p.m. with 2 c.c. of the "washings" (plasma in citrate normal saline solution after the removal of the corpuscles by centrifuging obtained from blood) taken on the second day of the sub-inoculated disease (Case 13).

3. v. 16. His temperature rose on the morning of this date to 99·6° F., and had increased to 100·6° at 5 o'clock in the afternoon. He was thereupon sent to hospital, although he protested that he had no symptoms and in fact never felt better in his life. On examination his face was flushed; the tongue not coated; the eyes somewhat injected; there was no coryza; the appetite was good. No rash.

4. v. He states that the right side of his head is aching badly and complains of pain in both heels and sleeplessness. Eyes injected; tongue coated; no rash; appetite good. Temperature, 8 a.m., 100·2°; noon, 101·2°; 8 p.m., 102·2°.

5. v. Feels weak but otherwise well. An erythematous blush on the forehead and right shoulder and in front of both knees. Temperature, 8 a.m., 100·2°; noon, 99°; 8 p.m., 101°.

Serum obtained from blood taken on this date and kept in the ice-chest for eight days, conveyed the disease by inoculation to Case 25. Serum obtained from blood taken on this day and mixed with similar serum from Case 16, was applied by scarification of the arm, by swabbing the nostrils, and by gargling and swallowing to Cases 22, 23 and 24 respectively, with negative or very doubtful results.

6. v. Complains of stiffness in the muscles but nothing else. No rash. Temperature, 8 a.m., 100·8°; noon, 100·8°; 4 p.m., 102°; 8 p.m., 102·2°; midnight, 102·8°.

7. v. Slight cough but no sputum; tongue coated. A raised pinkish mottling on both shoulders, on the back and in the region of the great trochanters, copious on the abdomen. Temperature, 4 a.m., 100°; 8 a.m., 100°; noon, 100°; 8 p.m., 101°.

8. v. Heavy sweating during the night. Eyes still injected; a reddish coated tongue. The rash was of the same character as previously, being prominent on the back and buttocks, copious on the abdomen, slight on the chest, shoulders and arms, and fairly copious round the lower ribs. Temperature, 8 a.m., 97·4°; noon, 99·4°; 8 p.m. 101·6°.

9. v. Sweating during the night. Rash fading.

10–11. v. Night sweating. Rash practically gone—most prominent round the lower ribs. Temperature subnormal on and after this date (taken till 16. v. 16).

12. v. Feels well; slight nocturnal sweating.

13. v. Feels well but weak; no sweating.

Remarks.

The incubation period was somewhat under six days to the onset of fever, seven days to the definite onset of symptoms. The temperature chart shews an irregular type of chart; the pyrexial period lasted a week and was interrupted by two definite intermissions. It may be regarded as an irregular saddle-back chart complicated by a secondary remission in the second pyrexial period due to the marked sweating which occurred. The pulse is for the most part relatively slow, and there are definite irregularly occurring periods of absolute bradycardia in the post-febrile phase. The recovery of the pulse in the later stages of convalescence is well shewn. The lowest pulse rate was 50. Sweating, as above mentioned, was a pronounced symptom in this case. It is perhaps worth noting that his temperature rose definitely a day before the onset of symptoms. Sub-inoculation of serum was successful.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood, taken on the sixth day of the Natural Disease (Case D), kept on ice four days, filtered, outside body seven days. *Result*: negative.

Case 18. *J. T., m.*, 64, was injected subcutaneously on 28. iv. 16 at 7.45 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate from the diluted blood of Case D. This blood was taken on the sixth day of a severe attack of dengue and was kept on ice for four days, filtered and was outside the body seven days from the time of inoculation. This man remained well. His temperature was taken once daily for twenty-seven days and was only normal or subnormal, except once when it was 98·8° F. (twentieth day).

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood, taken on the fourth day of the Inoculated Disease (Case 11), two days outside the body. *Result*: negative.

Case 19. *E. McS.*, was injected at 8 p.m. on 28. iv. 16 with 2 c.c. of a Pasteur-Chamberland filtrate of the blood from Case 11, taken on the fourth day of the disease and kept two days outside the body.

29. iv. 16. His arm was swollen and painful, and the temperature at 8 p.m. was 101° F.; midnight, 100·4°.

30. iv. Arm still swollen and sore. Temperature, 4 a.m., 99·8°; 8 a.m., subnormal; noon, 98·6°; 8 p.m., 99°.

1. v. Arm better; some areola. Temperature, 4 a.m., 99·2°; 8 a.m., subnormal.

2. v. Arm is well. Temperature normal.

The patient's temperature was taken four hourly until 10. v, and once daily until 23. v. 16, without shewing any increase above the normal, and no symptoms occurred.

The temperature of 101° F. on the morning following the injection was almost certainly due to some toxic body in the injected material, probably unconnected with dengue.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood taken on the second day of the Inoculated Disease (Case 12), outside the body eight days. *Result*: Negative.

Case 20. *J. P., m.*, 36, was injected subcutaneously on 4. v. 16 at 6.10 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate of Case 12, the blood being taken on the second day of the inoculated disease (26. iv. 16) and kept in the ice-chest for eight days. He remained well. On 6. v. 16 his temperature was recorded as 99·6°, but taken once daily for fifteen days after this it did not rise above normal.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood taken on the second day of the Inoculated Disease (Case 13), outside the body eight days. *Result*: negative.

Case 21. *W. F., m.*, 63, was injected subcutaneously on 4. v. 16 at 6.30 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate from blood taken on the second day of the inoculated disease (Case 13) and kept in the ice-chest for eight days. His arm became swollen and sore the next morning, and his temperature went up reaching a maximum at 5 o'clock in the afternoon of 100° F. This pyrexia was attributed to the presence of toxic bodies in the filtrate, probably independent of the virus of dengue.

The temperature was further taken until 3. vi. 16 (thirty days), and during that period he shewed occasional periods of pyrexia, viz. on 18. v. 16 to 99.3°, and on 27. v. 16 to 100·8°. These were considered as being independent of the injection of the filtrate.

Scarification of the Arm, as for ordinary vaccination, and application of mixed Serums from Cases 16 and 17, taken on the third day of the Inoculated Disease, two days outside the body. *Result*: negative (?). *Incubation period* (if any): seven days.

Case 22. *A. C., m.*, 19, was vaccinated as with calf lymph on the arm with the mixed serums of Cases 16 and 17, taken on the third day of the inoculated diseases, two days outside the body, on 6. v. 16, and was sent into hospital for observation on 13. v. His maximum temperature on this date was 98·8° F.

14. v. 16. Complains of headache; otherwise no other symptoms or signs. Temperature subnormal.

15. v. Feels well; no rash. Temperature subnormal.

16. v. Face a little flushed; eyes slightly injected; no rash but a congestion erythema of the back and erythematous flushes on the buttocks (pressure). Temperature at noon rose to 99·4°; 4 p.m., subnormal.

17. v. Tongue clean. A doubtful faint mottling on the back and thighs. The temperature at 4 p.m. rose to 99°; afterwards it was subnormal.

18. v. Diarrhoea; tongue slightly coated; suggestion of a mottled rash on the back, chest and abdomen. Temperature normal or subnormal on this date and subsequently.

19. v. Feels well; no rash.

20–23. v. Feels well; no rash.

24. v. Feels well, except for aching eyes.

25. v. Allowed up.

Urine (undated), 1020, alkaline, no albumen or sugar.

Remarks.

The incubation period, if any, would be seven days. The highest temperature shewn was 99·4° F. and the lowest pulse rate 52. There seems to have been some reaction in this experiment but so slight that the case must be accepted with due reserve. The vaccination gave no local reaction.

Nostrils swabbed with the mixed Serums of Cases 16 and 17, taken on the third days of the Inoculated Diseases, two days outside the body. *Result*: negative.

Case 23. *R. K., m.*, 48. The mixed serums from Cases 16 and 17 obtained from blood taken on the third days of the inoculated diseases, kept in the ice-chest two days, were applied by swabbing to each nostril on 6. v. 16. The patient was sent into hospital for observation on 13. v. 16.

14. v. 16. No symptoms complained of. On examination, there were no signs. The maximum temperature was 98·8° F.

15–16. v. No symptoms complained of. On examination, there were no signs. The maximum temperature was 99°.

16. v. Urine clear, 1015, acid, no albumen or sugar.

17. v. Feels well; tongue slightly coated; skin reaction to pressure very definite on the back, buttocks and shoulders. Suggestion of a pinkish mottling on the back, chest and abdomen. Temperature normal.

18. v. Face slightly flushed; eyes injected; tongue coated; rash (?), same distribution. Temperature normal on and after this date.

19. v. Feels well; rash (?) the same.

20. v. Feels well; rash (?) faint on the back and chest.

21. v. Feels well; rash (?) faint on the back and chest.

22. v. Feels well. Allowed up. Urine 1025, acid, no albumen or sugar.

Remarks.

There was no certainty as to the presence of a rash in this case, and the general reaction, if any, was so slight that the case must be considered as negative.

The Throat gargled with a mixture in milk, tinted with liquorice, of the mixed Sera of Cases 16 and 17, obtained from blood taken on the third days of the Inoculated Diseases, two days outside the body, the mixture being then swallowed. *Result*: positive (??). *Incubation period* (if any): seven days.

Case 24. *m.*, 30. On 6. v. 16 gargled his throat with and then swallowed a mixture in milk tinted with liquorice of the mixed sera of Cases 16 and 17, obtained from blood taken on the third days of the inoculated diseases, and kept for two days in the ice-chest.

13. v. 16. Owing to the presence of a fine punctate rash on the back, chest and both arms, he was sent into hospital. He complained of no symptoms. Temperature at noon, 98·6° F.

14. v. Complains of headache; rash fainter; no flushing of the face. Temperature all day normal or subnormal.

15. v. No symptoms; tongue coated; eyes slightly injected. Temperature at 4 p.m., 98·8°.

16. v. Temperature at noon rose to 100·4°, but normal before and after this.

17. v. Pains in the legs. A morbilliform rash on the chest, the abdomen and back; raised pinkish patches on the buttocks and thighs; pink erythematous slightly raised patches on the elbows; tongue coated and furred. Temperature normal or subnormal.

18. v. Pains in the right arm; strange in manner (the patient is epileptic); face slightly flushed; morbilliform rash fading. Temperature, 99° at 8 a.m., otherwise normal.

19. v. The morbilliform rash gone; pinkish definitely raised patches on the buttocks and the back; nil on the chest, abdomen, legs and arms.

20. v. Rash on back fading.

21. v. Rash faded; feels well.

Remarks.

The incubation period, if any, would be about seven days. This case may possibly be considered as a mildly positive one. There were practically no symptoms but the character of the rash, the bradycardia (lowest pulse reading 52), and the presence of slight fever (100·4° F. on 16. v. 16) support this view.

The patient insisted on his discharge on 22. v. 16 so we were unable to ascertain by inoculation whether any immunity had been established. An analysis of the urine was not obtained.

Subcutaneous Injection of Serum, obtained from blood taken on the third day of the Inoculated Disease (Case 17), seven days outside the body. *Result*: positive. Inoculation into this, a fourth individual, successful. Relapse. *Incubation period*: nine days.

Case 25. *L. J., m.*, 52, was injected at 7.45 p.m. on 12. v. 16 with (amount not stated) of the serum obtained from blood taken on the third day of the inoculated disease (Case 17), kept in the ice-chest for seven days. He became suddenly ill about 7 p.m. on 21. v. 16 with headache, dizziness and pains in the legs. The temperature at midday was 97·6° F.; the evening temperature was not taken.

22. v. 16. Face flushed; tongue clear; eyes slightly injected; no coryza; no rash. Temperature, noon, 102°; 4 p.m., 103°; 8 p.m., 103°; midnight, 103°.

23. v. Sleeplessness; no headache. A faint punctate rash on the back and abdomen. Temperature, 4 a.m., 102·2°; 8 a.m., 101°; noon, 100·2°; 8 p.m., 98·6°.

24. v. Pains in the lumbar region; eyes injected; sleeplessness. A faint rash on the back. Temperature, 4 a.m., 99·6°; 8 a.m., 99·6°; 8 p.m., 100°.

Blood, used as a gargle and then swallowed, taken on this date failed to convey the disease to Case 26.

25. v. Feels well but suffering from sleeplessness. A faint pinkish mottling in the lumbar region and on the thighs. Temperature, 8 a.m., 98·4°; 8 p.m., 99°.

26. v. Rash. Pinkish mottled patches on the chest and abdomen, particularly in the lumbar area and on the sides of the chest; more definitely raised pinkish masses on both thighs (symmetrically placed). Temperature, 8 a.m., 98·4°; 8 p.m., 98·6°.

27. v. Slept better. Rash the same. Temperature normal from this date until 5 . vi . 16.

28. v. Rash disappearing; slightly present still on the back and legs.

29. v to 2. vi. Feels well. Rash disappearing.

3. vi. Rash gone; allowed up.

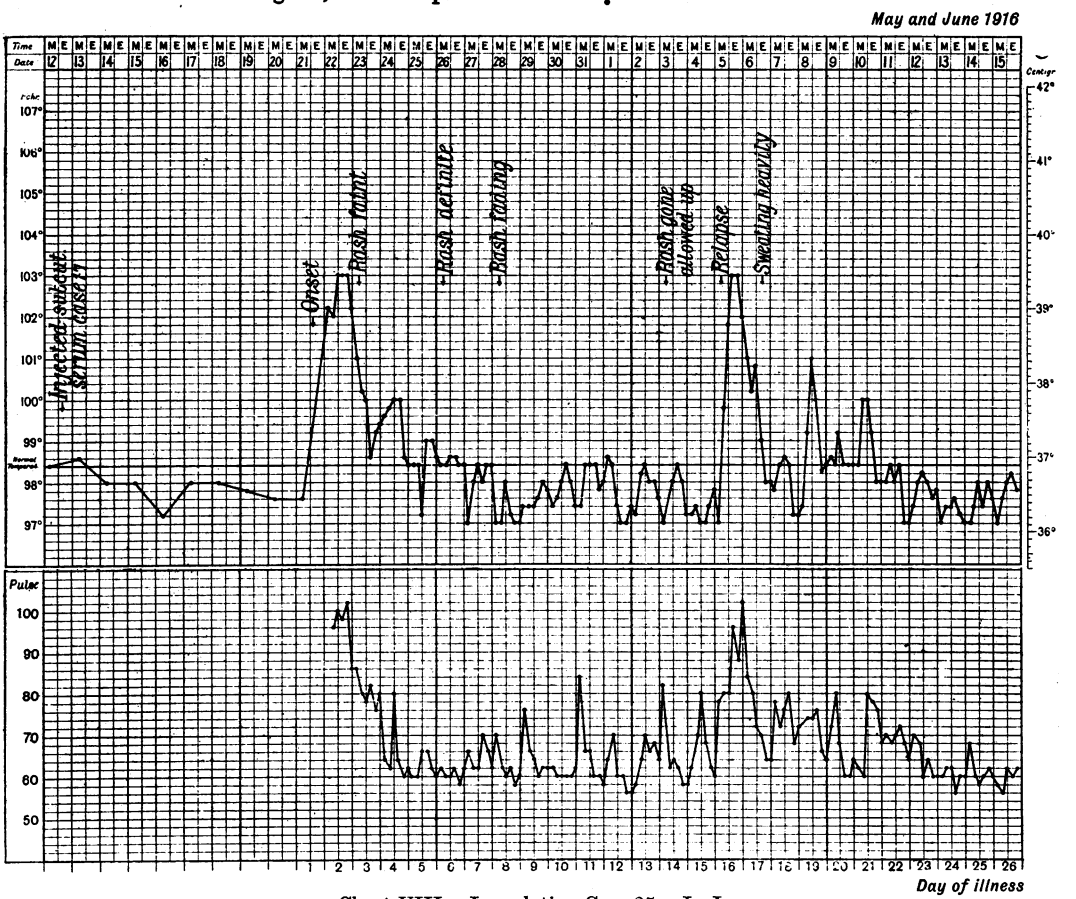


Chart VIII. Inoculation Case 25. L. J.

Relapse? The patient had been walking about for a few days, feeling well, when he became suddenly ill with very severe headache and a high temperature. At noon on 5. vi. 16 his temperature rose to 99·8° F., at 4 p.m. it was 101·8°, and at 8 p.m. and midnight 103°.

6. v. On examination: face flushed; eyes watery. Temperature, 4 a.m., 102°; 8 a.m., 101°; noon, 100·2°; 4 p.m., 100·8°; 8 p.m., 99°; midnight, 98°.

7. vi. Slight headache; slept well; copious night sweats (patient says three or four pints). Temperature normal or subnormal.

8. vi. Feels well; no sweating; no rash. Temperature at noon, 99·2°; 4 p.m., 101°; falling at 8 p.m. to 100°; normal at midnight.

9. vi. Feels well. Temperature reached 99·2° at 4 p.m. (maximum).

10. vi. Slight cough. Temperature reached 100° at midday and at 4 p.m. was 99°; at 8 p.m. and thereafter during convalescence normal or subnormal.

11. vi. Slight sweat and cough. Urine 1016, alkaline, no albumen or sugar.

13–15. vi. Feels well; slight cough.

16. vi. Feels well.

21. vi. Urine clear, 1015, neutral, no albumen or sugar.

The urine tested on two other previous occasions during the course of this case shewed: (A) 1010, acid, no albumen or sugar. (B) 1016, alkaline, no albumen or sugar.

Remarks.

The incubation period of the first attack is nine days, and a relapse occurred fifteen days later. The chart shews a single temperature curve during the first attack, and a typical double curve, with a period of apyrexia of two days, during the relapse. The pulse shews considerable relative and occasional absolute bradycardia. We have described this case as consisting of a single pyrexial period followed fifteen days later by a relapse, but one might consider the two phases as an example of an exaggeration of the two-phase characteristic with a very prolonged intermission.

Blood taken on the fourth day of the Inoculated Disease (Case 25), used as a Gargle and then Swallowed. *Result*: negative. Subsequently, Subcutaneous Inoculation of Serum and Corpuscles, obtained on the third day of the Mosquito-conveyed Disease (Mosquito Case No. 5). *Result*: positive. *Incubation period*: eight days, twelve hours.

Case 26 (32). *G. D., m.*, 67, on 24. v. 16 gargled his throat with and then swallowed a mixture composed of milk tinted with liquorice and blood taken on the fourth day of the inoculated disease (Case 25), used immediately. At 8 p.m. on 2. vi. 16 he was injected with ·5 c.c. of serum and corpuscles taken on the third day of the mosquito-conveyed disease ("B. B." Mosquito Case No. 5), outside the body several hours. He remained perfectly well and free from any symptoms until 2. vi. 16.

2. vi. 16. Headache; coated tongue; appetite good; face flushed; congestion erythema on the back. Maximum temperature, 98·8°.

3. vi. Feels well. Maximum temperature, 98·8°.

4. vi. Flushed face; pharynx injected; eyes watery; feels well; no rash. Temperature normal.

5. vi. Feels well. Maximum temperature, 99°.

6. vi. Maximum temperature, 99°.

7. vi. Some pains in the arms. Temperature normal.

8. vi. Feels well. Temperature normal.

9. vi. Feels well. Urine 1030, acid, no albumen, Fehling's solution reduced.

10. vi. Feels well. Temperature normal.

11. vi. He became definitely ill at 8 a.m. with headache and aching eyes; the tongue was coated; the eyes injected; the face flushed; there was no rash. Urine 1025, acid, no albumen, Fehling's solution reduced. Temperature normal.

12. vi. Headache and pains all over the body especially in the arms and shoulders. In the afternoon he complained of pains in the knees and more intense headache and aching eyes. Temperature, 8 a.m., 98·6°; 12 noon, 100°; 2 p.m., 102·6°; 4 p.m., 102·4°; midnight, 99·6°.

13. vi. Headache; faint mottling on the chest and abdomen. Temperature, 4 a.m., 100·2°; 8 a.m., subnormal; noon, 99·4°; 8 p.m., 99°.

14. vi. Faint mottling on the chest, back and abdomen. A heavy night sweat. Temperature normal after this date.

15. vi. Faint rash on the back, chest and abdomen.

16. vi. Feels well. Rash the same as on the previous day; itchy.

17–19. vi. Rash still the same—pinkish irregular mottled patches on the chest, abdomen and upper arms, doubtfully raised.

20. vi. Feels well. Rash the same. Urine 1017, acid, no albumen or sugar.

21–23. vi. Feels well. Rash on the back.

23–26. vi. Feels well. Rash on the back.

Remarks.

The incubation period is eight days twelve hours from the date of the injection to the onset of symptoms, and a little over nine days till the first rise of temperature. The reaction to the swallowing was very slight, if any, and must be considered as a negative result.

The reaction to the injection was very definite and was certainly positive (compare appearance, symptoms, rash and temperature).

An interesting feature was that the patient complained of typical symptoms twenty-eight hours prior to any elevation of the temperature.

The temperature chart shews a single-phase febrile paroxysm. There is marked relative bradycardia and the pulse rate tends to be slow throughout. It will be noticed, however, and we shall have more to say in this connection in discussing the pulse in these cases, that there is quite definitely a tendency to slow pulse well before the beginning of the attack.

The result of analysing the urine in this case was very surprising—the specific gravity on 9. vi. 16 being 1030, and a reduction with Fehling's solution taking place. Tested on 11. vi. similar results were obtained, but on 20. vi. the specific gravity was 1017 and there was no reduction of Fehling's solution.

Subcutaneous Inoculation of Blood taken on the second day of the Mosquito-conveyed Disease (Mosquito Case No. I), outside the body four days. *Result*: positive. *Incubation period*: seven days twenty-one hours.

Case 27. *H. K., m.*, 50, was injected subcutaneously on 24. v. 16 at 3 p.m., with 0·5 c.c. of the blood of J. G. (Mosquito Case No. I), taken on the second day of illness (20. v. 16) and kept in an ice-chest for four days.

This patient became suddenly ill after dinner (midday) on 1. vi. 16, suffering with headache and aching limbs. Temperature, morning, 98·4° F.; afternoon, 100·2°.

2 and 3. vi. 16. On examination: flushed face; coated tongue; watery eyes; no coryza; loss of appetite; sleeps well; feels "shivery all over." No rash. Temperature on morning of 2. vi. 16, 98·4°; 8 p.m., 99·6°; midnight, 100°. Temperature, 3. vi. 16, 8 a.m., 100°; noon, 100·6°; 4 p.m., 102·8°; 8 p.m., 100·6°; midnight, 102°.

4. vi. Headache severe; feels "shivery"; tongue coated; flushing of the skin of the back; no rash. Temperature, 8 a.m., 100°; 4 p.m., 102·8°; 8 p.m., 101°; midnight, 102·2°.

5. vi. Headache; aching in shoulder muscles; eyes still watery; tongue clearing; no rash. Urine clear, 1018, acid, no albumen or sugar. Temperature, 4 a.m., 99°; 8 a.m., subnormal; noon, normal; 4 p.m., 99°; midnight, 100·2°.

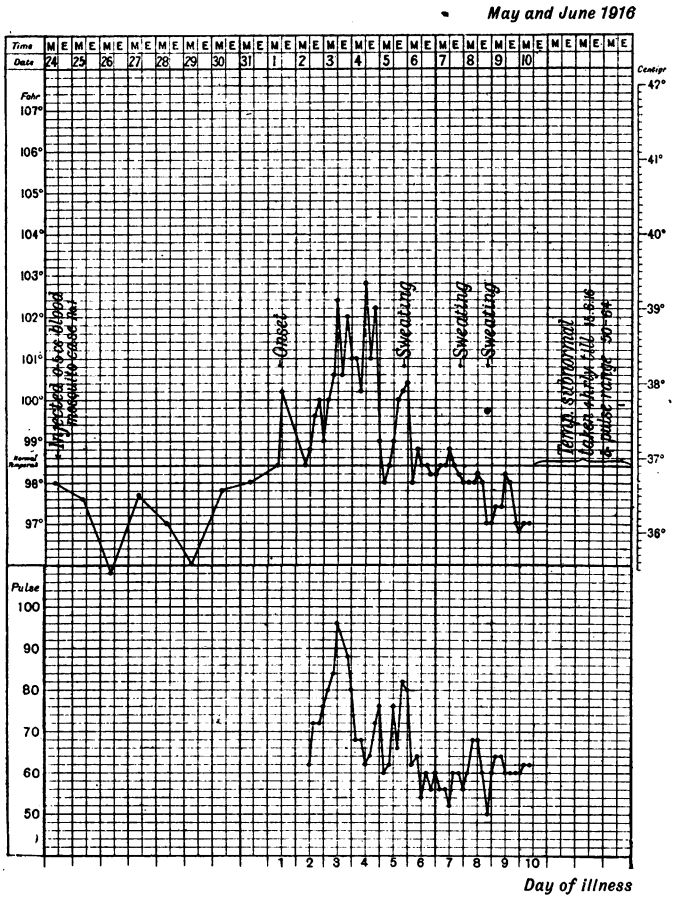


Chart IX. Inoculation Case 27. H. K.

6. vi. Headache; aching in the shoulder muscles; heavy sweating the previous night. Temperature, 4 a.m., 100·4°; 8 a.m., subnormal; midday, 98·8°; afternoon, normal.

7. vi. Feels warm; eyes watery; tongue clearing. Highest temperature at 4 p.m., 98·8°; temperature normal after this.

8. vi. Heavy sweat during the previous night; feels weak; eyes watery.

9. vi. Heavy sweat during the previous night; dizziness; slept well.

10–13. vi. Feels well; eyes still watery; appetite poor; no rash.
14. vi. Aches in the back of the head.
15. vi. Discharged.
19. vi. Urine clear, 1020, acid, no albumen or sugar.

Remarks.

The incubation period is nearly eight days (seven days twenty-one hours). The temperature chart shews an irregular chart with two remissions before the final fall. The pulse shews definite relative and, later on, some absolute bradycardia. The lowest pulse recorded was 50. There was no rash. During convalescence the patient complained of marked general weakness.

Subcutaneous Inoculation of Blood, taken on the fourth day of the Mosquito-conveyed Disease (Mosquito Case No. I), outside the body two days. *Result*: negative (?).

Case 28. *N. M.*, 63, was injected subcutaneously on 24. v. 16 with 1 c.c. of the blood of J. G. (Mosquito Case No. I), taken on the fourth day of illness (22. v. 16). His temperature was taken once daily—on the fourth, eighth, ninth, eleventh and twelfth days it is recorded as being 99° F. On the fifteenth day the temperature was 99·2° in the morning and 102° in the afternoon; on the sixteenth day 100° in the morning and 102° in the afternoon; on the seventeenth day 100° in the morning and 100·8° in the afternoon; on the eighteenth day 99·2° in the morning, and 101° in the afternoon. After this, the temperature, taken once daily until the twenty-second day, was normal.

There are no notes in this case of any symptoms like those of dengue.

Subcutaneous Inoculation of Blood, taken on the second day of the Mosquito-conveyed Disease (Mosquito Case No. III), two days outside the body. *Result*: positive. *Incubation period*: five days twenty-one hours.

Case 29. *P. S., m.*, 46, was injected subcutaneously on 24. v. 16 at 3 p.m. with 1 c.c. of blood from W. (Mosquito Case No. III), taken on the second day of illness (22. v. 16).

The patient states he felt unwell, feeling drowsy and having aching eyes, about 1 p.m., on 29. v. 16. He took to his bed about 6 p.m. on 30. v, his temperature being 101° F.

31. v. 16. He complains of pain in the head, back, thighs and hips, and sleeplessness. On examination: the face was slightly flushed; the eyes injected; the tongue coated; no coryza; no cough; no rash but a congestion erythema of the back. Temperature, 8 a.m., 100°; 4 p.m., 101°; midnight, 100·4°.

1. vi. Marked headache; aching eyes; pains all over the body; the pharynx injected; slight cough; a pinkish raised erythematous mottling, irregularly arranged around little islets of white, was copious on the back, chest, abdomen, buttocks, thighs and shoulders. Temperature, 4 a.m., 99·8°; 8 a.m., 101·2°; noon, 102·2°; 4 p.m., 102·8°; 8 p.m., 102°; midnight, 100·6°.

2. vi. No symptoms; tongue coated; cough; vomiting during the previous night. Rash the same as on the previous day. Temperature, 4 a.m., 99·3°; 8 a.m., 101·2°; noon, 100°; 8 p.m., 99·8°; midnight, normal.

3. vi. Feels well; profuse sweating during the previous night; rash still copious

on the back, chest, shoulders, abdomen and thighs. Temperature, 8 a.m., 98·8°; 4 p.m., 99·4°; midnight, normal.

4. vi. Feels weak; rash fading except on the shoulders. Temperature normal.

5. vi. Feels well; rash has disappeared except from the shoulders. Temperature, normal in the morning, rising to 99° at midnight; after this date it was normal.

6. vi. Still weak. Rash has disappeared except from the shoulders.

7 and 8. vi. Still weak. Rash has disappeared except from the shoulders.

9. vi. Still weak. Rash has disappeared except from the shoulders.

10. vi. Feels well. Rash has disappeared except from the shoulders.

11 and 12. vi. Feels well. Rash has disappeared except from the shoulders. Slight sweating on the night of 11 . vi . 16.

15. vi. Feels well. Rash still on the shoulders, but it was faint on his discharge on this date.

Remarks.

The incubation period was about five days to the first symptom and just over six days to the time he took to bed. The temperature chart is irregular and shews two intermissions. The pulse shews relative bradycardia in the febrile stage and later tends to be slow. The lowest pulse reading was 56. This case was definitely positive.

Subcutaneous Inoculation of Serum and Corpuscles from blood taken on the third day of the Mosquito-conveyed Disease (Mosquito Case V), inoculated the same day, into a subject who had recovered from the inoculated disease (Case 13). *Result*: negative.

Case 30. Case 13, who had reacted positively to dengue material injected on 18. iv. 16, becoming ill on 24. iv, and being discharged on 9. v, was reinjected subcutaneously on 2. vi. with 0·5 c.c. of serum and corpuscles from Mosquito Case No. V ("B. B.") taken the same day, the third day of B. B.'s illness. Although this blood has produced a typical positive result in Case 26, no result followed the re-injection in this case. The temperature was taken once daily for fourteen days. Only on one day (6. vi.) was the temperature above normal, being on that day 99·6° in the morning and 99·2° F. in the afternoon.

The result of the reinjection was negative.

Subcutaneous Inoculation of Serum and Corpuscles from blood taken on the fourteenth day of the Mosquito-conveyed Disease (Mosquito Case No. V), outside the body several hours. *Result*: negative.

Case 31. *G. R., m.*, 49, was injected subcutaneously on 13. vi. 16 with eight minims of serum and corpuscles from Mosquito Case No. V ("B. B."). This blood had been taken the same day, the fourteenth day of B. B.'s illness. No symptoms followed. The case was under observation at least nine days and the temperature taken once daily did not rise above the normal.

Editorial Note

Of the numerous charts sent by the authors a limited number have been reproduced, typical ones being selected. Owing to their absence on military service the authors were unable to pass the proofsheets of this publication.