

FURTHER EXPERIMENTS IN THE ETIOLOGY OF DENGUE FEVER.

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(With 9 charts.)

*(From the Laboratories of the Department of Public Health and of the Royal
North Shore Hospital of Sydney.)*

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INTRODUCTION.

IN a previous communication (1), after reviewing the literature of the subject, and especially the claims as to the transmission of dengue by *Culex fatigans*, we were able to show conclusively that in Australia the Yellow Fever mosquito, *Stegomyia fasciata*, is capable of transmitting the virus of dengue. Whether or not other mosquitoes can also play a part we cannot say, although epidemiologically we think this unlikely. We also showed that the incubation period was certainly over five days, and usually between five and a half and nine and a half days, in our mosquito cases. We showed also that the virus could be transmitted by the subcutaneous inoculation of blood.

In a further communication (2) we showed *inter alia*:

1. That the virus of dengue may be present in the whole blood, the serum, the washed corpuscles or the citrated plasma.
2. That the virus was present in the blood on the second and third days of the disease (24 to 48 hours after onset), and possibly as late as the eighth day (about 168 hours after onset). Some evidence was adduced to show that the virus was not present in the blood on the fourteenth day of the disease.
3. That our filtration experiments were not conclusive. There was one apparently successful case out of five experiments. In some of these, for other reasons, the result might have been expected to be negative.
4. That the incubation period varied from six to nine days.
5. That immunity to inoculation was present twenty-four days after recovery from dengue.
6. That blood containing the virus may retain its infectivity in a cool place outside the body for at least seven days.
7. That the infection of dengue could be conveyed to at least four "generations" of artificial (injected) cases.

The present series of experiments is the outcome of a project which to a great extent has not materialised. We had intended to establish a chain of artificial dengue cases in volunteers, to keep the virus alive in the series by successive injections, and with this virus to perform as it were a series of side-chain experiments on other volunteers to obtain certain special aspects of the problem. We had made arrangements to breed out mosquitoes and had devised experiments to confirm our previous findings and to determine several important points as to the nature of the transportation of the virus by these insects. We had also planned to conduct experiments as to the nature of the virus in man, and especially as regards its filtrability, its resistance to outside influences, its persistence in the blood and its distribution in the elements of the blood.

Certain facts combined caused us to greatly curtail our scheme and eventually the virus-chain ran out and further experiments were abandoned. This was chiefly due to the unexpected difficulty of obtaining volunteers, even with a considerable monetary inducement. The mosquitoes which we were able to breed for some reason did not bite well and died off rapidly.

This should be borne in mind in considering the experiments herein described. At first sight, in several instances, we seem to have neglected opportunities for experiments, whereas in reality we had no volunteer available at the time.

Again, in two experiments, the finding of a positive complement fixation reaction for syphilis in the volunteer prevented further utilisation of the virus in his blood.

In spite of these difficulties we have been able to obtain further information on certain points as to the dengue virus.

The present experiments show in brief:

1. That it is possible by subcutaneous injection of blood, or derivatives of blood, from a dengue patient to transmit the disease to a healthy non-immune, and from this case in

a similar way to derive a series of experimental cases. In the present communication the original virus obtained from a natural case has been passed successively through four "generations" of artificial cases, with a doubtful positive case in the fifth "generation."

2. That the virus under certain conditions may be passed through a Pasteur-Chamberland F. filter which at the same time is keeping back ordinary organisms (staphylococci, colon bacilli).

3. That the virus does not appear to be specially contained in any one element of the blood. Carefully washed corpuscles, citrated plasma, and serum free from corpuscles all contain the virus. It is not absolutely certain, however, in the case of serum that the virus may not obtain access to this by the breaking up of corpuscles.

4. That the virus is resistant to conditions outside the body for several days (99 hours).

5. That in the blood of the dengue patient the virus was present in one case 18 hours after the onset, in one case 67 hours and in another as late as 90 hours, whilst several cases show it to have been present at 57 hours and less after the onset. It was not found in a case 130 hours after the onset.

6. That the incubation period, while variable and not shorter than $4\frac{1}{2}$ days, is usually from 6 to 8 days, but may be as long as 15 days.

7. That immunity may be present to injection 229 days after the onset of a previous attack of dengue.

8. That, in one experiment made, no evidence could be found that *Culex fatigans* can transmit the virus of dengue.

9. That, in one experiment made, no evidence was found that the blood of a guinea-pig contained the virus seven days after its inoculation.

10. That no result followed inoculation of guinea-pigs or rabbits, and no pathological lesions were found in inoculated guinea-pigs and no spirochaetes were found in their organs by Levaditi's method.

PART I. INJECTION EXPERIMENTS.

Reviewing the cases of the present series, certain peculiarities are shown which may be briefly summarised.

The type of the disease, as in fact is the case in the natural illness, varied very greatly. Certain of our cases were typical of dengue, whilst others were only recognisable as such by the temperature and pulse variation and the nature of the surrounding circumstances. A striking phenomenon is the mildness of the symptoms in the aged volunteers from the Asylum, in contrast to the general severity of the cases in the younger and more active volunteers from our staff and from that of the Royal North Shore Hospital. As will be mentioned later, mild symptoms were often associated with a very definite pyrexia.

Cases 2, 4, 15 and 17 were all classifiable, as regards symptoms, as of "typical rather severe" type. That is to say, they all had marked general body and eye pains and headache with considerable prostration, nausea and malaise, and all looked ill. The rashes in these cases were a prominent feature, and complete recovery was slow, probably in all. Now it is extremely interesting to note that whereas Cases 2 and 4 are at the beginning of our series, Cases 15 and 17 are both fourth generation cases and are separated from the two severe early cases by comparatively mild cases, Case 15 being derived

from Case 10, and Case 17 from Case 11—both Case 10 and 11 being descendants of Case 4. In Case 10, for instance, the volunteer only complained of symptoms for one day and the rash was extremely faint, whereas the descendant Case 15 was feeling very sick for three days, and had a most distinct rash and typical symptoms.

Case 17 was of considerable severity with a distinctly typical rash, whereas Case 11, although showing definite though slight symptoms and a definite slight rash, was altogether much milder than Case 17.

The febrile reaction in most cases was distinct and constituted in some the chief evidence as to the presence of the infection. Some of the cases were not charted in detail but in most cases we have a detailed record of the temperature and pulse from the date when the virus was administered to some time after the end of the febrile phase. The course adopted in this series was to take the temperature twice daily, usually for a week, and if no symptoms or pyrexia declared itself in this period to take the chart four-hourly for the remaining period. The total period of observation was usually between three and four weeks.

A review of Table I and of the charts will indicate, better than a description, the type, duration, and severity of the febrile reaction. It will be seen that the febrile period ranges from somewhat over three days to seven days. In most cases it is easy enough to determine the point of onset of the pyrexia, as also its termination, but occasionally there may be a slight elevation of temperature to about 99° F. for a day or more before or after the termination of the definite fever.

The degree of fever: A review of the maximal points in nine of the positive charted cases shows the lowest temperature to be 100·8° F. and the highest 104·6° F. 102° F. would be about the average maximum temperature and in four cases it lies between 102–103° F. The fever seems hardly at all correlated with the symptoms—in some of our cases, especially in Case 14, the want of correlation is especially distinct. This particular case had virtually no symptoms with a temperature of nearly 105° F., which remained high for nearly two days, then fell and rose again to nearly the same level with another short interruption, and then fell abruptly.

Type of fever: Although several of the charts are definitely saddle-back in type, and others show a more irregular diphasic variation, others again show an irregular temperature curve. We do not think the type of temperature is at all diagnostic of Australian dengue, either in natural or artificial cases. Although certain cases may show typical saddle-back charts, and as may be seen in our previous papers there may be two separate pyrexial phases, yet other typical cases may show a simple monophasic variation or irregular or plateau types of chart.

The pulse and its relationship to the pyrexia: As shown in our previous papers and noted by other observers in natural cases, the pulse of dengue has a distinct tendency to be slow relatively to the degree of fever. This may

Table I.

Case No.	Initials	Result	No. of days during which temp. taken	Incub. period in days	Pyrexia		Type	Highest temp. °F.	Duration in days	Days charted after Pyrexia	Pulse during Pyrexia.		Remarks
					Charted	Abs.					Rel.	Abs.	
2	W. G. A., m.	Pos.	15	8	Irreg.	102.8°	6	1	+	+	++		
3	B. B., m., 34	Neg.	Not taken	Immune	
4	H. G., m., 40	Pos.	Not taken	6½	...	About 102°	++		
5	P. N., m., ?	Neg.	33	...	(See history)	Doubtful case.	
6	H. D., m., 45	Pos.	28	15	Irreg. diphasic	100.8°	7	6	+	+	++		
7	W. J. I., m., 72	Neg.	21		
8	J. W. M., m., 63	Pos.	23	10	Irreg. diphasic	101°	7½	6	+	-	+ s		
9	J. W., m., 65	Neg.	22		
10	P. M., m., 45	Pos.	23	9	Irreg. diphasic	102.6°	7	7	++	-	+ s	Rises to 99° after pyrexia	
11	J. D., m., 74	Pos.	21	7	Plateau	102.4°	3 +	11	++	-	+	Later oscillations to 99°	
12	P. B., m., 61	Neg.	23		
13	R. C., m., 58	Pos.	20	8?	Irreg.	100.8°	6?	7	++	+	-	Temp. 99° one day before onset	
14	H. W., m., 70	Pos.	17	5	Typical saddleback	104.6°	7	5	++	-	+ s	Whole chart not shown	
15	W. M., m., 30	Pos.	12	7	Incomplete chart, prob. irregular	102°	4	1	-	-	++		
16	A. A., m., ?	Neg.	21	7		
17	N. K., f., 28	Pos.	...	?	No chart	100.3°		
18	J. F. H., m., 70	Pos.	19	4½	Irreg.	100.8°	5	9	++		
19	T. B., m., 55	Neg.	23		
20	C. L., m., 70	Pos.?		
21	H. McD., m., 42	Neg.	Not charted		

be accompanied by absolute bradycardia (pulses between 50 and 55 are fairly frequent, especially after the pyrexia). Although several of our charts in this series show periods when the pulse is between 50 and 55, definite absolute bradycardia is not a very prominent feature. On the other hand the

relative bradycardia is present in every case of which we have complete charts, and in some of these is very distinct.

Rashes: No attempt was made in this series to separate primary and late rashes and detailed descriptions of the rashes are not given. Where it occurred it corresponded to our previous description and was typical of dengue and not readily to be confused with that of any other complaint. In the Asylum cases the rashes were slight, but in the cases among the younger more active volunteers from this staff and elsewhere the rashes were very prominent and characteristic.

Out of eleven cases which were regarded as positive, five showed distinct and typical rashes; one showed a definite, but less distinct, rash, whilst two cases showed only a slight rash which was not prominent enough to be noticed unless specially sought for. The other cases, although they showed an erythematous flushed skin, could not be said to have a rash at any period of their illnesses.

Symptoms: These need not be detailed here. Reference to the Appendix will show that they were prominent and typical in the outside cases but mild, sometimes practically absent, in the institution cases. The experience referred to above, in which mild cases intervened in the series between severe cases, teaches us to attach less importance to symptoms in our class of institution volunteers and to rely more on the temperature-pulse variation for determining the positivity of the cases.

INFORMATION BEARING ON THE NATURE OF THE VIRUS, ETC., TO BE OBTAINED FROM THE CONSIDERATION OF THE CASES IN THIS SERIES.

Table II indicates the relationship of the human inoculation experiments in this series; it records briefly the nature of the injection, date of injection, date of onset, and the incubation period.

Certain special aspects of the experiments are discussed here (see Appendix, where full experimental and clinical data are recorded).

Transmissibility of dengue: As we and others have shown that dengue fever can be successfully transmitted by the blood of a person with the fever to a non-immune and on through several generations, this matter need not be elaborated here. In the present series the virus has been transported through four "generations" of artificial cases. The type and severity of these cases have been discussed above.

Time during which the blood contains the virus: Table II shows the duration of the illness in the previous case at the time the material was taken, the period spent by the virus outside the body, and the nature of the material used for injection, correlated with the results of the experiments. This table shows that the virus was present in the blood of the dengue patient as late as 90 hours after the onset in one case; 67 hours in one case; 57 hours in three cases; 47 hours in two cases; 46 hours in one case; 22 hours in two cases; and 18 hours in one case.

Therefore these cases demonstrate that the virus is present in the blood as early as 18 hours after the onset of symptoms and may be present as late as 90 hours after. As we have only one case showing this we cannot be certain that this long period may not be exceptional. However, between 60 and 70 hours after the onset the virus is probably usually present in the blood.

As regards injections followed by negative results, only two of these can be fairly used as indicating an absence of infectivity of the blood. The remaining cases might for other reasons have given a negative result. Case 21, which was negative, injected with the blood of an atypical case taken only a few hours after the onset, will be specially discussed. Cases 12 and 16 were injected with untreated blood. The virus used for the injection of Case 12 was outside the body less than 24 hours and that of Case 16 only a few hours. In Case 12 the blood was taken from Case 4 on the sixth day of the illness (approximately 130 hours after the onset). In Case 16 the blood was derived from Case 10 on the ninth day of the disease (approximately 190 hours after the onset). Both Case 4 and Case 10 were shown to have had the virus in the blood at an earlier stage. From these two cases it may be deduced that the virus was not present in the blood at 130 hours and 190 hours after the onset.

Summary: The present figures show that the virus may be present in the blood as early as 18 hours and as late as 90 hours after the onset. It was not found in the blood 130 hours and 190 hours after the onset.

Resistance of dengue virus outside the body. The virus seems to resist well for a short period the conditions of a sojourn outside the body. As far as possible when preserving blood for injection we have made use of an ice-chest but in several cases the material was transferred between the laboratories and the hospital with no special precautions, and filtration was performed at room temperature without destroying the virus.

In one instance the untreated blood was preserved, chiefly in the ice chest, for 99 hours before injection and gave rise to a typical case (Case 8). There were several instances where the virus was outside the body about 48 hours, and in one case after 72 hours the virus was possibly still active (doubtful Case 20).

Summary: The virus outside the body has been found alive, if kept reasonably cool, in several instances up to 48 hours, and in one instance after 99 hours.

It is not possible from this series to draw deductions from the negative cases on this question.

Filterability of the virus. In our last communication we reported one apparently positive case and several negative cases after filtration. The single positive case was not fully controlled. In the present series we obtained four negative and three positive results (see Table II).

As regards the negative results, Case 19 can be excluded as the blood was taken, from Case 11, 115 hours after the onset, and our experiments suggest that after this period the virus may have disappeared from the blood.

Table II. *Showing sequence of experiments.*

(1) Dr F. G. (natural case). Ill, 11-1-17 (evening). Blood taken, 13-1-17 (4 p.m.)	(3) Dr B. B., m., 34 yrs. Inj. 0.5 c.c. dil. blood, 15-1-17 (4 p.m.). Result— <i>Negative</i> .
(2) Dr W. G. A. Inj. 0.5 c.c. dil. blood, 15-1-17 (4 p.m.). Ill, 23-1-17, 5 p.m. Incubation period, 8 days. Result— Positive .	(4) H. G., m., 40 yrs. Inj. 1 c.c. diluted blood, 24-1-17 (2.30 p.m.). Ill, 31-1-17, 3 a.m. Incubation period, 6½ days. Result— Positive .
Blood taken, 24-1-17 (11 a.m.)	Blood taken, 27-1-17 (11 a.m.)
(5) P. N., m. Inj. 9 c.c. diluted filtered blood, 25-1-17 (? 4.30 p.m.). Result— <i>Negative</i> .	(6) H. D., m., 45 yrs. Inj. 0.5 c.c. blood, 27-1-17 (5.30 p.m.) Ill, 11-2-17. Incubation period, 15 days. Result— Positive .
Blood taken, 31-1-17 (about 11 a.m.).	Blood taken, 2-2-17 (noon).
(7) W. J. I., m., 72 yrs. Inj. 2 c.c. diluted filtered blood, 1-2-17 (4.30 p.m.). Result— <i>Negative</i> .	Blood taken, 5-2-17 (noon?). (12) P. B., m., 61 yrs. Injected 0.3 c.c., 6-2-17 (10 a.m.). Result— <i>Negative</i> .

<p>(8) J. W. M., m., 63 yrs. Inj. 0.8 c.c. blood, 6-2-17 (3 p.m.). Ill, 16-2-17 (2 p.m.). Incubation period, 10 days. Result—Positive.</p>	<p>(9) J. W., m., 65 yrs. Inj. 2.8 c.c. blood filtrate, 3-2-17 (4.30 p.m.). Result—Negative.</p>	<p>(10) P. M., m., 45 yrs. Inj. 1.3 c.c. washed corpuscles, 3-2-17 (4.30 p.m.). Ill, 12-2-17 (5 p.m.). Incubation period, 9 days. Result—Positive.</p>	<p>(11) J. D., m., 74 yrs. Inj. 2 c.c. washings, 3-2-17 (4.30 p.m.). Ill, 10-2-17 (4.30 p.m.). Incubation period, 7 days. Result—Positive.</p>
<p>Blood taken, 13-2-17 (3 p.m.).</p>			
<p>(13) R. C., m., 58 yrs. Inj. 2.3 c.c. plasma filtrate, 15-2-17 (4 p.m.). Ill, 23-2-17 (4 p.m.). Incubation period, 8 days. Result—Positive.</p>	<p>(14) H. W., m., 70 yrs. Inj. 0.3 c.c. blood, 15-2-17 (4 p.m.). Ill, 20-2-17 (3 p.m.). Incubation period, 5 days. Result—Positive.</p>	<p>(15) W. W., m., 25 yrs. Inj. 2 c.c. serum filtrate, 16-2-17 (10 a.m.). Ill, 23-2-17 (on rising). Incubation period, 7 days. Result—Positive.</p>	<p>(16) A. A., m. Inj. 1 c.c. blood, 20-2-17 (5 p.m.). Result—Negative.</p>
<p>Blood taken, 15-2-17 (about noon). Blood taken, 20-2-17 (time not noted).</p>			
<p>(20) C. L., m., 70 yrs. Inj. 1.2 c.c. blood, 27-2-17 (7 p.m.). Incubation period (?). Result—Doubtful.</p>	<p>(17) N. K., f., 28 yrs. Inj. 2 c.c. (?) blood filtrate, 14-2-17 (time not noted). Ill between 21 and 26-2-17. Incubation period, doubtful. Result—Positive.</p>	<p>(18) J. F. H., m., 70 yrs. Inj. 2.6 c.c. washings, 14-2-17 (3 p.m.). Ill between 18 and 19-2-17. Incubation period, about 4½ days. Result—Positive.</p>	<p>(19) T. B., m., 55 yrs. Inj. 2.3 c.c. serum filtrate, 18-2-17 (10 a.m.). Result—Negative.</p>
<p>Blood taken, 12-2-17 (3 p.m.).</p>			
<p>Blood taken, 15-2-17 (about noon).</p>			

Table III.

Showing the data concerning materials used for injection in relation to the results obtained.

Case	Result	Incubation period in days	Materials injected			Hours outside body†	Rash	Remarks
			Nature and source	Hours after onset*	Hours outside body†			
(1) Dr F. G., m.	Pos.	Over 5	Naturally acquired	46	48	++		
(2) Dr W. G. A., m.	Pos.	8	0.5 c.c. (1 to 2) diluted blood from Case 1	46	48	++		
(3) Dr B. B., m., 34 yrs.	Neg.	...	Same as No. 2	46	48	-	Immune	
(4) H. G., m., 40 yrs.	Pos.	6½	1 c.c. diluted (1 to 1) blood from Case 2	18	3½	++		
(5) P. N., m.?	Neg.	...	9 c.c. diluted (1 to 9) blood, filtered, from Case 2	18	About 29	-		
(6) H. D., m., 45 yrs.	Pos.	15	0.5 c.c. undiluted blood from Case 2	90	6½	++	Congenital syphilis	
(7) W. J. I., m., 72 yrs.	Neg.	...	2 c.c. diluted (1 to 7) blood, filtered, from Case 4	8	30	-		
(8) J. W. M., m., 63 yrs.	Pos.	10	0.8 c.c. undiluted blood from Case 4	57	99	++?		
(9) J. W., m., 65 yrs.	Neg.	...	2.8 c.c. diluted (1 to 3) blood, filtered, from Case 4	57	28	-		
(10) P. M., m., 45 yrs.	Pos.	9	1.3 c.c. washed corpuscles from Case 4	57	28	++?		

(11)	J. D., m., 74 yrs.	Pos.	7	2 c.c. diluted (1 to 5) citrated plasma from Case 4	57	28	+
(12)	P. B., m., 61 yrs.	Neg.	...	0.3 c.c. undiluted blood from Case 4	130	<24 hours	-
(13)	R. C., m., 58 yrs.	Pos.	8?	2.3 c.c. diluted (1 to 4) citrated plasma, filtered from Case 10	22	49	-
(14)	H. W., m., 70 yrs.	Pos.	5	0.3 c.c. undiluted blood from Case 10	22	49	-
(15)	W. W., m., 30 yrs.	Pos.	7	2 c.c. undiluted serum, filtered, from Case 10	67	22	++
(16)	A. A., m.	Neg.	...	1 c.c. undiluted blood from Case 10	About 190	A few hours	-
(17)	N. K., f., 28 yrs.	Pos.	?	? 2 c.c. diluted (1 to 5) blood, filtered, from Case 11	47	About 48	++
(18)	J. F. H., m., 70 yrs.	Pos.	4‡	2.6 c.c. diluted (1 to 2½) citrated plasma from Case 11	47	48	-
(19)	T. B., m., 55 yrs.	Neg.	...	2.3 c.c. diluted (1 to 1) serum, filtered, from Case 11	115	70	-
(20)	C. L., m., 70 yrs.	?	?	1.2 c.c. undiluted blood from Case 15	24	About 72	-
(21)	H. McD., m., 42 yrs.	Neg.	...	2 c.c. undiluted blood from Case 20	?24	48	-
(22)	P. W. P., m., 71 yrs.	Neg.	...	2 c.c. undiluted blood from guinea-pig 3647	See animal experiments		-

* Hours after onset.—This refers to period elapsing between onset of illness in previous (donor) case and time at which blood was taken from this patient.

† Outside body.—This refers to period elapsing between the time the blood was drawn from donor case and the time when it was injected into the recipient case.

In Case 5 there was some doubt as to whether the result was really negative but there was not a characteristic enough reaction to say that the case was positive. The blood at the time it was taken for filtration was shown to contain the virus (see Case 4). The virus was only outside the body 29 hours.

In Case 7 there was no control to show that the virus was present at the time in the untreated blood from Case 4, but three cases injected with other material taken two days later from the same case (whose blood was used for the filtrate) were positive. It is probable that at the earlier period (8 hours after onset) the blood of Case 4 did contain the virus. The failure in Case 7 cannot therefore be reasonably explained by supposing an absence of the virus at that time in Case 4. The blood was only outside the body 30 hours, and this therefore again cannot be held to account for the failure. The subject will be further considered later.

In Case 9, three cases serve as controls to show that the virus was present in the blood when it was withdrawn from Case 4, and in two of these the virus was outside the body as long as it was in the material used for Case 9 (28 hours) while in the third case the virus was outside the body for a much longer period (99 hours) and was still successfully conveyed.

In the three positive filtration cases—13, 15 and 17—material was taken 22, 67 and 47 hours respectively after the onset of the “mother” cases and was outside the body 49, 22, and about 48 hours respectively.

Before considering the causes determining the failure or success of individual cases we may dwell on the technique of filtration.

Technique of filtration.

The filter used was a small candle about six inches long with a wall one-eighth to one-sixteenth of an inch thick at the top, the inside diameter at the top being ca. one-third of an inch. The filter tapered slightly from top to bottom. It was marked “Chamberland *Sme.* Pasteur B.E.S.G.D.C.H.B. & Cie, Choisy-le-Roi BES S.G.D.B.,” and stamped “Contrôle,” and on the bottom “F.” It is what is known as the Pasteur-Chamberland F. Filter, which is said to be a coarser grade than the B. type.

For filtration, previously unused candles were fixed in bored rubber corks into the neck of flasks which had side tubes. The whole apparatus was sterilised by steam and cooled, and then attached by the side tube, which was plugged with cotton wool, to the rubber pipe leading to a water pump. The cork was covered with melted paraffin to be sure no leak occurred. The material to be filtered was run into the candle and the pump turned on. When sufficient material had been obtained the plug was removed from the side tube, the end of this flamed and cooled, or wiped with alcohol, and the flask tilted and the filtered material run out into a sterile tube. Generally speaking the pressure at the Health Department Laboratory is not good and the later filtrations were done at the Royal North Shore Hospital Laboratory.

To show that the filters used did not admit the passage of ordinary bacteria, the practice was adopted of mixing organisms with the material to be filtered and testing for their presence by culture in the filtrate.

The organisms added and the nature of the cultural tests were as follow:

Case 5, injected with filtered blood from Case 2 with a doubtful result.

Organism added. Emulsion of *B. coli communis* L.I.P.M.

Cultures. 1. Broth culture, 1 c.c. of filtrate to 20 c.c. broth. Agar subcultures made from this.

2. Broth cultures, one loop and two loops.

3. Agar cultures, one loop and two loops.

Case 7, injected with filtered blood from Case 4 with a negative result.

Organism used. Colon bacillus (*B. acidi lactici* type).

Cultures. 1. Broth cultures, one and two loops and subcultures from these.

2. Agar cultures, one and two loops.

Case 9, injected with filtered blood from Case 4, with a negative result.

Organism used. *B. coli communis* L.I.P.M.

Cultures. Broth and agar.

Case 13, injected with filtered plasma from Case 10, with a positive result.

Organism used. *B. coli* (type not noted).

Cultures. 1. Two loops into 10 c.c. broth.

2. One loop on agar.

Case 15, injected with filtered serum from Case 10, with a positive result.

Organism used. *Staphylococcus aureus*.

Cultures. 1. Two loops into 10 c.c. broth.

2. One loop on agar.

Case 17, injected with filtered blood from Case 11, with a positive result.

Organism used. Colon bacilli (type not noted).

Cultures. Two loops into 10 c.c. broth.

Case 19, injected with filtered serum from Case 11, with a negative result.

Organism used. *Staphylococcus aureus*.

Cultures. 1. Two loops into broth.

2. One loop on agar.

All the cultures in this series remained sterile. The number of organisms added to the material before filtration was large but not specially measured. When an emulsion was used it was made densely milky. In other cases a large loopful of thick scrapings from the cultures was used. For full details of this work see under "Experimental Material," following the record of the case from which the material was derived.

Although in future experiments we would be inclined to use larger amounts of the filtrate than two loops for making the cultures, yet we think in view of the number of organisms added that the above tests show fairly clearly that our filters were restraining the passage of ordinary bacteria under the conditions of the experiments. We think the method of adding the organism to the material to be filtered is a much better control than testing the filter before or afterwards, as, with a variable water pressure, it may not be possible to parallel the conditions of the actual filtration. Moreover the filter itself may be altered in some way by washing and sterilising if controlled before the main filtration, and may be blocked by debris from the filtration if the bacterial test is left until afterwards. No serious effects followed the injection

of any of the filtrates, though a slight rise of temperature and some redness of the arm (possibly due to toxins from the bacteria) were noted next day in some cases.

FINAL CONSIDERATION OF THE RESULTS OF THE FILTRATION EXPERIMENTS.

Our results indicate that the dengue virus under certain conditions passes through the Pasteur-Chamberland F. Filter, which at the same time is preventing the passage of ordinary bacteria. The question now arises as to what is the cause of the failure in certain experiments.

Confining ourselves to the present series of tests, Case 19 may be rejected as a test as to the filtrability of the virus for reasons given above. In the negative cases, 5, 7 and 9, however, the virus was almost certainly present in the blood on withdrawal and in Cases 5 and 9 was actually shown to be present in the unfiltered blood from which the filtrate was made.

The sojourn outside the body less than 30 hours in any of the cases, is less than in two of our positive filtration cases and in several of our non-filtration positive cases, so that it is at first sight hard to find any ground for the failure of these cases. It is most unlikely that they were all immunes.

The three unsuccessful cases were obtained with filtered blood which before filtration contained a certain amount of solid material and the filtration was done in the Department's laboratory, and although we have no precise notes on the matter, the filtration was certainly slower than in the later cases, including three successful filtrations. Of the later cases, although in Case 13 filtration was also performed at the Department's laboratory, the filtration took place rapidly, the fluid being free from cellular material. In Cases 15, 17 and 19 filtration took place under better pressure conditions at the North Shore Hospital and filtration was more rapid.

We therefore conclude that the failure of certain of our cases was due to slow filtration and the plugging of the filter pores by solid material through which the fluid had to pass, and the successful cases were due to the more rapid filtration brought about by a higher water pressure and an absence of solid material.

Distribution of the virus in the blood.

Graham (3), whose results have not received confirmation, described endo-corporuscular bodies in dengue fever. These we regard as artefacts. The fact that the virus has been shown to be filtrable, although it shows that at some stage the virus is very small, does not of course exclude the possibility of an endo-corporuscular phase of a microscopically visible size. Although we have examined blood from dengue cases carefully a number of times with unstained preparations, and with the ultramicroscope, we have not found any evidence of a visible virus. Apart from the question, however, of a visible virus it would be useful if possible to show whether the causative agent was confined to any one element of the blood. It is well to remember, however, that the

distribution may not be the same at all stages. The organism may at one stage, for instance, be endo-corpuseular or endo-leucocytic, and at another stage free in the serum. On the other hand the virus may be a special inhabitant of one or other element of the blood.

Our previous results led us to think that, whatever be the nature of the virus, it was apparently not strictly endo-corpuseular, though we could not exclude the possibility of an endo-corpuseular or endo-leucocytic phase followed or accompanied by a phase in which it was free in the plasma. Our present series of experiments, though not yet completely demonstrative, tend to support our previous view.

It should be remembered, however, that processes designed to effect a separation of the blood elements may at the same time cause some breaking up of the corpuscles and a consequent liberation of a virus. A consideration of the whole circumstances, however, leads us to think that this theoretical breaking up cannot explain the results and that, whether or not the virus is solely endo-corpuseular or endo-leucocytic at some stage, it was present in the plasma in the cases examined.

Cases 10, 11, 13, 15 and 18 of the present series may be considered in this connection. For full details of the preparation of the materials injected we refer to the antecedent cases.

Cases 10 and 11 may be first considered together. Case 10 was injected with 1.3 c.c. of four-times washed corpuscles (see Case 4), and Case 11 with the corpuscle-free washings from the same case from blood taken at the same time. Both Cases 10 and 11 were definitely positive.

The 2 c.c. of washings in Case 4 would roughly correspond to the plasma obtained from 0.3 c.c. of the original blood. This dose is small. The amount of corpuscles injected into Case 10 on the other hand was rather large. These two cases seem to us rather to support the contention that the virus is free in the plasma. The large dose of corpuscles used, and the probably great difficulty of freeing such material from adherent virus, may be the explanation of the success with the corpuscles. We unfortunately had no further opportunity of checking these results by injecting more nearly equivalent doses of the two materials.

The other experiments all concern the presence of virus in the fluid element (serum or plasma), and tend to support the view that the virus is naturally free in the fluid element.

Case 18 was injected with "washings" from Case 11, with a positive result. The dose of washings (which showed some haemolysis) was 2.6 c.c., which would be equivalent to the plasma from 0.5 c.c. of the original blood.

Cases 13 and 15, although not specially designed for the purpose, support the finding of virus in the fluid part of the blood. In Case 13 we obtained a positive result after injection of 2.3 c.c. of filtered citrated plasma from Case 10. The dose corresponds approximately to the plasma from 0.23 c.c. of the original blood. Haemolysis was not noted. In Case 15, 2 c.c. of filtrate

from the serum of Case 10 produced a positive result. This serum filtrate was distinctly haemolysed and the dose (2 c.c., undiluted) was large.

THE INCUBATION PERIOD.

Our previous results showed that the incubation period of dengue varied between 5 and $9\frac{1}{2}$ days. We found no incubation periods as short as those described by Ashburn and Craig(4), and by Graham(3). The present series confirms the view that the incubation period is usually about a week, but we have a greater variation in the periods than we encountered previously. However, our shortest period is still considerably longer than the incubation period found by these other observers in Syria and the Philippines.

The incubation periods observed in the successful cases were as follows: 8, $6\frac{1}{2}$, 15, 10, 9, 7, 8, 5, 7, $4\frac{1}{2}$ days (in Case 17 the exact period could not be determined).

The shortest incubation period was actually about 4 days 19 hours (Case 18), and the longest almost exactly 15 days.

There does not seem to be any relation between the time the virus was outside the body and the incubation period, nor between the duration of illness of the previous case and the incubation period.

The cause of the variation is not known.

Immunity.

There is only one experiment bearing on immunity, namely, Case 3. In this instance the subject of the experiment was injected at the same time, with an approximately equal amount of the same material, as was used to inoculate Case 2 (a non-immune). Whereas Case 2 developed typical symptoms, Case 3 had at no time subsequent any signs of the disease.

Case 3 was the subject of mosquito experiments referred to in our earlier communication(1) and had passed through a typical attack of rather severe dengue starting on 31. v. 16. On 15. i. 17, at 4.15 p.m., Case 3 was inoculated subcutaneously with about 0.5 c.c. of diluted blood from Case 1, and thereafter showed no signs of the disease. Thus 7 months and 15 days (229 days) after the onset of an attack of dengue immunity was present to a dose of virus, which brought about a typical and rather severe result in the control case.

PART II. MOSQUITO EXPERIMENTS.

Although a number of experiments were attempted with various species of mosquitoes, *Stegomyia fasciata*, *Culex fatigans*, *Culicella vigilax*, *Culicella annulirostris* and *Scutomyia notascripta*, most of these were failures because the mosquitoes died out or would not bite, or because volunteers were not available. Special experiments with *S. fasciata*, hatched from larvae brought from Mullumbimby, similarly failed. The following experiments, however, are quoted in detail.

With Culex fatigans. 22 ♀♀ and 1 ♂ of this species were collected in Sydney and enclosed in a cage with a muslin sleeve. They were allowed to feed on Cases 10 and 11 on 15 and 16. II. 1917, and bit both patients well. Case 10 was thus bitten three or four days after the onset, and Case 11 five and six days after the onset. Case 10 was shown to have virus in the blood a few hours before the biting on 15. II. (see Case 15). Case 11 was bitten rather late and probably the blood at this time did not contain any virus. Many of the mosquitoes were alive on 18. II., but could not be counted owing to the nature of the box. On 18. II. and each night until 26. II. (inclusive) the mosquitoes remaining alive bit Volunteer Mrs W. G. A. No result followed. Although it is not possible to say exactly how many mosquitoes bit either the donor or recipient, it is certain that both were bitten by the mosquitoes in the cage.

The experiment tends to support previous experiments which failed to demonstrate that *Culex fatigans* is capable of transmitting dengue.

With Culicella vigilax. About 40 of this species were caught at Berowra on 15. II. 1917, and on the same day bit Case 10 (three days after the onset). Again on 16. II. (four days after the onset) at midday he was bitten. The mosquitoes also bit Case 11 on the same day (five days after the onset) and on 16. II. (six days after the onset).

There were only about a dozen mosquitoes left alive when on 17. II. they were fed on a volunteer, H. McD.—one of the insects bit. After this but few were alive and none bit although tested daily until 21. II. for periods of 30–45 minutes.

No results followed. The experiment is inconclusive.

Summary. Several mosquito experiments in this series have not therefore added to our previous knowledge, but the one satisfactory experiment with *Culex fatigans* tends to support the view we hold that this species is probably not a vector of the disease. The results previously published by us support the opinion of Bancroft (5) and show clearly that, whether or not *C. fatigans* may also play a part in Australia, *Stegomyia fasciata* is capable of spreading the infection of dengue.

PART III. ANIMAL EXPERIMENTS.

An Attempt to Transmit the Virus through a Guinea-pig to a Human Being with a Negative Result.

On 13. II. (at 3 p.m.) blood was taken from Case 10 (22 hours after the onset). This blood was shown, by the successful injections of Cases 13 and 14, to contain the virus.

On 13. II. (afternoon) a guinea-pig, No. 3647, was injected subcutaneously with 0.25 c.c. of blood (serum and corpuscles). The animal remained well and on 21. II. (morning) it was bled to death, and at noon on the same day 2 c.c. of the blood (serum and corpuscles) were injected into Case 22, P. W. P.,

m., 71 years. His temperature and pulse were taken daily for four days, and four-hourly for 14 days.

On 25. II. at 10 a.m. (four days after the injection) the temperature reached 99° F., and was on the same level during part of the day of 26. II., but this was unaccompanied by any other signs or symptoms, and the temperature after this remained normal.

There is no evidence, therefore, of the survival of the dengue virus after 7½ days in a guinea-pig or of its multiplication in this animal.

OTHER ANIMAL EXPERIMENTS.

These need not be separately detailed as they were uniformly negative.

Guinea-pigs and rabbits were injected intraperitoneally and subcutaneously without result, and sections from the organs of some of these were examined both by iron-haematoxylin and eosin stained sections and by Levaditi's method, and showed no abnormality. Our object in using Levaditi's method was in the hope of demonstrating possible spirochaete-like organisms and was undertaken in view of the results obtained with epidemic jaundice in France.

APPENDIX

DETAILED REPORT OF THE CASES DEALT WITH IN THE SERIES OF INJECTION EXPERIMENTS.

Case 1, Dr F. G. (naturally infected). This case forms the starting-point of the series of experiments with which this report is concerned.

The patient was on a holiday in Molangool, near Bundaberg, Queensland, where he states that occasional cases of dengue fever were occurring, and where mosquitoes (*Stegomyia fasciata*) were biting freely in the daytime. He left Molangool on 5. I. 17, passing the night in Bundaberg and thence journeying to Sydney by train. He arrived in Sydney on the evening of 7. I. 17. Thus his last day in Queensland, and the last day on which he was exposed to *Stegomyia* bites, was on 6. I. 17. He became ill on 11. I. in the evening, therefore the incubation period must have been at least five days, and, assuming he was infected in the Molangool district, six days or more.

He kindly volunteered to allow blood to be taken for our experiments, and accordingly one of us went to see him on the 13. I. at 4 p.m., and took about 1 c.c. of blood from the median basilic vein.

This case was apparently typical and fairly severe. He had the usual symptoms and a marked rash, which was very obvious on 13. I. He had not taken his temperature regularly and only stopped in bed one day.

Experimental material. The blood taken on 13. I. 17, at 4 p.m., ca. 46 hours after the commencement of the illness, was kept on ice until 15. I. at 4 p.m., when it was used to inject Cases 2 and 3. There was about 1 c.c. of clot and serum in the test-tube. This was shaken up with about 2 c.c. of sterile normal saline solution, and about 1 c.c. of the fluid part, containing

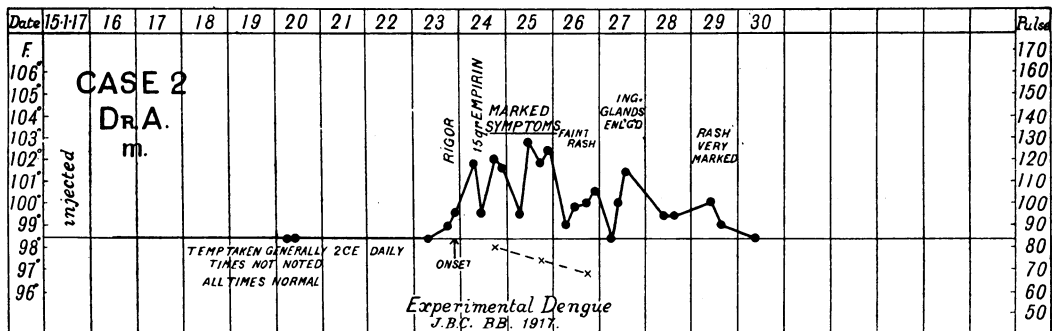
corpuscles, serum and saline, was sucked up into a syringe and equal amounts were injected into Cases 2 and 3.

Subcutaneous injection of 0.5 c.c. of diluted (1 to 2) blood taken from Case 1, 46 hours after the onset, virus kept outside the body 48 hours. *Result positive.*

Case 2, Dr W. G. A., m. 15. i. 17. On this date Dr A. of this Department volunteered for experiment, and at 4 p.m. he was injected subcutaneously with 0.5 c.c. of diluted blood from Case 1. The blood had been taken from Case 1 about 46 hours after the onset and had been outside the body for 48 hours; it was diluted ca. 1 to 2.

From this date the temperature, which was taken once at least and usually twice daily, remained normal until 23. i.

20. i. On this date he first felt slight rheumatic pains and some degree of languor on rising in the morning. The temperature was normal at 7 a.m.,



and at 9.30 a.m. There is no record of the evening temperature. After this there were occasionally some vague symptoms of languor, etc., but no rise of temperature until 23. i. Leucocytes 8500 per c.mm.

23. i. At 5 p.m. the temperature had risen to 99° F., and by 9 p.m. to 99.5° F. At 10 p.m. there was a rigor. A restless night was passed and he had occipital headache, painful eyes and vague rheumatic pains.

24. i. At 7.30 a.m. the temperature was 101.8° F. The patient came in to work and between rising and 11.30 a.m. took three 5-grain doses of aceto-salicylic acid. The temperature was 99.5° F. at 11.30 a.m. At midday he returned to his home. At 5.30 p.m. the temperature was 102° F. and the pulse 80, and at 9.30 p.m. the temperature was 101.6° F. At about 11 a.m. blood had been withdrawn from a vein and used to inject Cases 4 and 5. Blood from the ear and the serum from clotted blood from the vein were examined ultra-microscopically without finding any recognisable parasites.

25. i. He stopped in bed all day and had severe headache, pains in the limbs and eyes, and several chills. He took 5 grains of aceto-salicylic acid every three hours. The temperature at 7.30 a.m. was 99.5° F.; at 11.30 a.m.

102.8°; at 5.30 p.m., 101.8° (pulse 74); at 9.30 p.m., 102.4°. He passed a very restless night and did not sleep until 4.30 a.m.

26. I. A faint macular rash was discernible on the abdomen and thorax. The temperature ranged from 99° to 100.5° F. (see Chart).

27. I. The inguinal glands were distinctly enlarged on both sides. The temperature was 100° F. at 10.30 a.m., and 101.4° at 2.30 p.m. Leucocytes 3400 per c.mm. Blood was taken on this day about 11 a.m. from a vein and used to inoculate Case 6.

28. I. There was a faint but distinct morbilliform rash on the hands and forearms, most distinct on the palms. The temperature was 99.4° F. morning and evening.

29. I. The rash was very distinct and well-defined on the abdomen, chest, forearms, thighs, back, and palms of the hands. The face was free. The temperature was 99° to 100° F.

30. I. The temperature was normal and the patient felt practically well, although stiffness and a slight feeling of indisposition were present for some days afterwards.

Remarks. The case was a very definite one of dengue of rather severe type and the patient looked very ill for several days. The rash was extremely marked and very widespread. The incubation period, to the rise of temperature, was almost exactly eight days, but vague indisposition occurred just before five days had elapsed. Blood from this case, taken on the second day after the rise of temperature (24. I. 17), ca. 18 hours after the onset, gave a *positive result*, i.e. it induced the typical disease in Case 4, and blood taken on the fifth day of the disease (27. I. 17), or about 90 hours after the rise of temperature, caused the disease in Case 6.

Experimental material. (a) Blood was taken at 11 a.m. on 24. I. 17, ca. 18 hours after the onset as indicated by a rise of temperature. About 2.5 c.c. of blood was thus obtained and allowed to clot, and then ground up in a mortar with 2.5 c.c. of sterile normal saline solution.

1 c.c. of the more fluid part of this mixture was injected into Case 4.

1 c.c. of the same was injected into a guinea-pig, without result.

3 c.c. of the same was mixed with 2 c.c. of a saline emulsion of *B. coli communis* and filtered through a Pasteur-Chamberland F. candle and subsequently 10 c.c. of water were added. About 10 c.c. of red stained fluid came through fairly quickly. There remained in the filter candle about 5 c.c.

The filtrate was tested as follows:

On 24. I. 17, 1 c.c. was added to about 20 c.c. of broth. The original culture remained sterile up to 31. I., after which it was not further examined. Agar subcultures from this remained sterile. The last subculture was made on 31. I. and examined on I. II.

On 24. I. 17 broth and agar cultures were made with one or two loops of the filtrate. These remained sterile until the last examination on 31. I.

The remaining filtrate (ca. 9 c.c.) was used to inject Case 5.

Although the above figures for dilution are only approximately accurate, it will be observed that the blood used to inject Case 4 was diluted ca. nine times with saline and water (1 to 9).

(b) Blood taken at 11 a.m. (90 hours after the onset) on 27. I. was not treated in any way but was used to inject Case 6.

Injection of a volunteer who had had dengue eight months previously, with virulent dengue blood. *Result negative.*

Case 3, Dr B. B., m., 34 years. One of us, who had been the subject of experimental mosquito-borne dengue eight months previously, was injected on 15. I. 17, at 4 p.m., with 0.5 c.c. of the diluted blood from Case 1. The amount injected was approximately equal to that injected at the same time into Case 2.

On 16 and 17. I. there was some headache, sore throat and languor but no areola round the injection and the temperature was normal. No symptoms developed after this date.

The result was *negative*, but Case 2, similarly injected, but not protected by a previous attack of dengue, developed this disease.

Subcutaneous injection of 1 c.c. of about equally diluted blood and saline solution taken from Case 2, 18 hours after the onset, virus kept outside the body $3\frac{1}{2}$ hours. *Result positive.*

Case 4, H. G., m., 40 years. This volunteer was injected subcutaneously on 24. I. 17, at 2.30 p.m. with 1 c.c. of diluted blood serum and corpuscles from Case 2. The blood was taken from Case 2 at 11 a.m. on 24. I. 17 (18 hours after the onset). The dilution was with approximately equal parts of blood and sterile saline solution (for details see Case 2). The virus was outside the body about $3\frac{1}{2}$ hours.

The patient remained well until 31. I. although he complained of slight headache a day or so before the definite onset.

31. I. 17. He arrived at the Laboratory looking very sick and said he had been taken ill about 3 a.m. He had pains "all over" and headache and felt very weak. His face was flushed. There was no definite rash. The temperature was 100.4° F. and the pulse 120, on arrival at the Laboratory about 10 a.m. The leucocytes were 8700 per c.mm.

Blood was taken on this day at about 11 a.m. from the median basilic vein and, after filtration, was used for the injection of Case 7, with a *negative result.*

2. II. He stated that he had had two "bad" days at home, feeling sick, but was better now. There was a well-marked rash. Leucocytes 16,000 per c.mm.

Blood taken at midday from a vein was used as material to inject Cases 8—11, three of which were *positive.*

5. II. The patient felt fairly well.

Blood was taken at midday (?) and used to inject Case 12 with a *negative result*.

Remarks. The incubation period was $6\frac{1}{2}$ days. Although a temperature chart was not regularly taken, the case was absolutely typical and moderately severe. The rash was well marked on the trunk and arms. Inoculation with blood taken from this case, 31 hours after the onset, gave a *positive result*, reproducing the disease in several cases.

Experimental material. First specimen. A specimen of blood was drawn from a vein on 31. I. 17, at about 11 a.m., about eight hours after the onset. A little serum was separated for the Bordet-Gengou test for syphilis, which proved to be negative.

The remaining serum and clot were mixed with about equal parts of boiled tap water. Some of this mixture was removed with the object of using it subsequently for injection but when required it was found to have become contaminated. The remainder was again equally diluted with a watery emulsion of a colon bacillus (*B. acidi lactici* type) and crushed up as far as possible in a mortar. The more fluid portion was put into the filter but would not pass through the candle. A further addition of water of approximately equal amount to the already diluted material was added. Filtration was slow and only about 2 c.c. of filtrate were obtained. The dilution, when filtered, was about 1 to 7, but this is not accurate having regard to the removal of the more fluid part for the Bordet-Gengou reaction, etc., and the final discarding of the clot.

Cultures were made from the filtrate—one and two loops on broth, and one and two loops on agar, and subsequently subcultures were made on agar from the broth. All cultures remained sterile.

The whole filtrate, consisting of about 2 c.c. of fluid, was injected into Case 7 with a *negative result*.

Second specimen. This was taken from a vein on 2. II. 17, at midday, ca. 57 hours after the onset.

(A) 2 c.c. were squirted from the syringe into ca. 10 c.c. of 1.5 % sodium citrate in normal saline. This was centrifuged for 15 minutes and the supernatant fluid separated from the deposit.

The *supernatant fluid* was then centrifuged for one hour and the fluid, down to $\frac{1}{2}$ in. from the bottom of the tube, drawn off. This was labelled "*washings*," and contained no corpuscles microscopically. The dilution would be 1 to 5. Cultures on broth and agar made on 2. II. remained sterile. These "*washings*" gave a *positive result* in Case 11.

The *corpuscles* from the first centrifugalisation were suspended in sterile saline solution and shaken and recentrifuged for 15 minutes. The supernatant fluid from this centrifugalisation was drawn off, fresh sterile saline added, and the mixture again shaken and recentrifuged for 15 minutes. The procedure was repeated and the residual corpuscles were retained. Cultures made on

agar and broth remained sterile. These four-times washed corpuscles were labelled "*washed corpuscles*." They gave a *positive result* in Case 10.

(B) About 5 c.c. of blood-clot were broken up with a sterile wire, and 2 c.c. of the more fluid part were separated and labelled "blood"; 0.8 c.c. (14 minims) of this were used to inject Case 8, with a *positive result*.

(C) The remaining 3 c.c. of clotted blood were diluted with about 9 c.c. of a watery emulsion of *B. coli* L.I.P.M., and were filtered in the usual way. The filtrate was tested by agar and broth cultures and found sterile. This was labelled "*filtrate*," and 2.8 c.c. were used to inject Case 9 with a *negative result*.

Third specimen. Blood was taken on 5. II. 17 at ? midday, and without dilution, 0.3 c.c. (5 minims) of serum and corpuscles were injected into Case 12. With *negative result*.

Summary of experimental results obtained from material from Case 4.

Diluted (1 to 7) filtered blood taken about eight hours after the onset gave a *negative result* (Case 7).

Undiluted blood taken 57 hours after the onset gave a *positive result* (Case 8).

Washed corpuscles taken 57 hours after the onset gave a *positive result* (Case 10).

Diluted (1 to 5) washings taken 57 hours after the onset gave a *positive result* (Case 11).

Diluted (1 to 3) filtered blood taken 57 hours after the onset gave a *negative result* (Case 9).

Undiluted blood taken approximately 130 hours after the onset gave a *negative result* (Case 12).

Subcutaneous injection of 9 c.c. of 10 times diluted filtrate (1 to 9) from Case 2, taken less than 18 hours after the onset. Virus kept outside the body ca. 29 hours or less. *Result negative*.

Case 5, P. N., m. On 25. I. 17, this volunteer was injected, at a time not noted but probably about 4.30 p.m., with 9 c.c. of a filtrate prepared from blood taken from Case 2 on 24. I. 17, at 11 a.m., *i.e.* ca. 18 hours after the onset. The virus was thus outside the body probably about 29 hours. This dilute filtrate would correspond roughly to the fluid from 0.9 c.c. of blood. The arm was sore and showed an erythematous flush for a few days around the site of inoculation, and the temperature rose next day to 99° F. This rise was evidently due to toxins in the material inoculated, probably from the colon bacilli used as a test for possible permeability of the filter.

Examination of the temperature chart (not published), which was kept from 28. I. until 28. II. 17, and was taken four-hourly from 3 to 28. II., shows a rise to 99.4° F. on the ninth day, followed by several minor rises

above normal, until on the 22nd day the temperature reached 100° F. followed by 99·2° F. on the 23rd and 24th days of illness.

During the whole period that this chart was kept, the patient worked hard and felt well. His body was examined daily but showed no rash or other signs of infection. The pulse was variable, being usually rather slow and often at about 50 and sometimes lower.

Remarks. Taking all things into consideration, although it is possible that there may have been a modified reaction to the virus, the case must, for the purposes of proof of filtrability of the dengue virus, be regarded as *negative*. This experiment is discussed separately in the section dealing with filtrability of the virus.

Subcutaneous injection of 0·5 c.c. of undiluted blood from Case 2, taken 90 hours after onset. Virus kept outside the body 6½ hours.

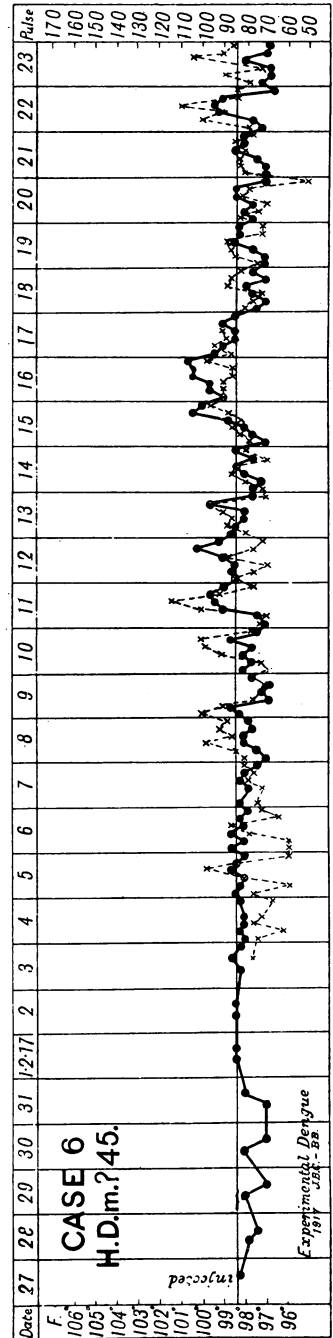
Result positive.

Case 6, H. D., m., 45 years. This case was injected at 5.30 p.m. on 27. I. 17 with 0·5 c.c. of untreated blood taken from Case 2 on 27. I. 17, at 11 a.m. The virus had been taken 90 hours after the onset and was outside the body 6½ hours.

This patient felt a little “out of sorts” on the afternoon of 11. II. 17, and had a rash on his shoulders with a temperature at 6 p.m. of 99·6° F.

12. II. 17. On examination the face was suffused, the eyes and pharynx injected, the tongue slightly coated, and a slight cough was present. There was a well-marked rash consisting of a pinkish raised papular erythematous mottling over the whole body, except below the knees and on the hands. The rash tended to run together into large patches. It was very copious in the armpits and groins. It began on the shoulders and quickly covered the upper three-fourths of the body.

13. II. The rash was more copious but



slightly duller in colour. The distribution was the same. The rash was seen by several medical men and photographed. The patient felt well.

14. II. The rash was fading. There was still some cough. The patient felt well.

15. II. The same as on 14. II.

16-17. II. The rash was only visible on the back. The patient felt well.

18-21. II. There was now only an erythematous blush on the back and no rash. The patient felt well.

23. II. The patient was allowed up.

Remarks. The chart of this case shows an irregular diphasic temperature variation with a late third rise. The incubation period, measured to the first definite rise of temperature, is about 15 days, which is double that of the usual period found in our first series of cases. However, the present series contains other instances of long periods and the case cannot be rejected as being positive on this account. The symptoms were very mild but the patient was of a very low mental type and it was hard to get intelligent replies to questions.

A specimen of blood was taken with the object of using it for further inoculations, but the case gave a strongly positive Bordet-Gengou ("compluetic") test and thus prevented this being done. The rash was in our opinion, and in that of others who saw it, definitely not a syphilitic manifestation and equally definitely was of the type seen in dengue. The case bore evidence of old, probably congenital, syphilis and no evidence of recent infection.

The temperature-pulse relationship, although not typical of dengue as seen in some of the charts, shows for the most part a relatively slow pulse in relation to the height of temperature.

Subcutaneous injection of 2 c.c. of filtered blood from Case 4, the blood taken eight hours after the onset in Case 4. Virus kept outside the body 30 hours. *Result negative.*

Case 7, W. J. I., m., 72 years. On 1. II. 17, at 4.30 p.m., the volunteer received subcutaneously 2 c.c. of the filtrate from Case 4. The blood from which this was obtained was taken on 31. I. 17, eight hours after the onset, and was kept outside the body for 30 hours. The temperature was charted for 22 days twice daily and, except for a rise to 99° F. on the day after the injection, remained normal.

Remarks. This case gave a definitely *negative result* with filtered diluted blood. There was no control case injected with untreated blood taken on this date, but blood taken on the third day of Case 4 gave *positive* results, and therefore it is almost certain that blood taken on the first day was infective and that the failure of the filtrate to produce infection cannot be attributed to an absence of virus in the blood.

This case is discussed fully in the section on filtrability of the virus.

Subcutaneous injection of 0.8 c.c. of untreated blood taken from Case 4, 57 hours after the onset. Virus kept outside the body 99 hours. *Result positive.*

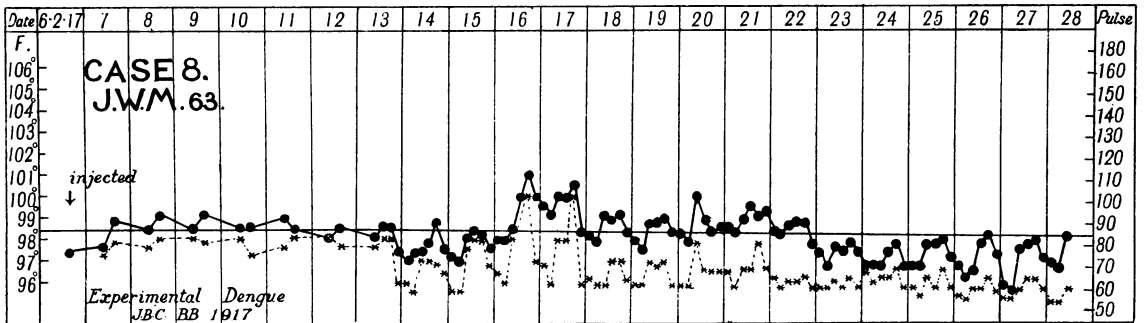
Case 8, J. W. M., m., 63 years. On 6. II. 17, at 3 p.m., the case received subcutaneously 0.8 c.c. of blood which had been withdrawn from Case 4 on 2. II. 17 at noon, that is, 57 hours after the onset, and kept on ice for 99 hours.

The temperature, as can be seen on the chart, was slightly above normal for several days after the injection, but between 11. II. and 15. II. it remained practically normal or subnormal.

16. II. When the patient entered the ward at 2 p.m. to have his temperature taken, he looked flushed, and his temperature was found to be 100° F., but he did not complain of any symptoms. That evening the temperature rose to 101° F.

17. II. He was examined in bed: face flushed, eyes clear, tongue not coated, pharynx injected, slight cough but no coryza. He stated he felt well. There was an erythematous flushing of the back. Maximum temperature 100.5° F.

18-19. II. No rash present, tongue coated. Temperature was 99.2° F.



20. II. Tongue clearing, a faint mottling on the abdomen and back. Maximum temperature 100.2° F.

21. II. Mottling on chest, back and abdomen, but not on legs and arms. The patient felt well. Temperature 99.7° F.

27. II. Patient allowed up. The rash was fading. Temperature had been subnormal since 23. II.

Remarks. The incubation period was ten days. The temperature variation was roughly diphasic having its maxima at 6 p.m. on 16. II. and at 10 a.m. on 20. II. The pulse tended to be slow and this bradycardia is noticed on the chart to start several days before the definite onset. During the first period of pyrexia the pulse was on two occasions raised correspondingly with the temperature, but for the most part and especially in the later stages of the pyrexia remained relatively slow, until the end of the temperature-taking.

Although slow, there was a noticeable correspondence between the oscillations of the pulse and those of the temperature, a correspondence which is seen in several cases. The most striking feature was the entire absence of symptoms. There can be little doubt, however, that this case was one of mild dengue infection.

The patient gave a partially positive Bordet-Gengou (compluetic) reaction, and therefore further inoculations could not be practised.

Subcutaneous injection of blood filtrate from Case 4. The blood was taken 57 hours after the onset and was kept outside the body 28 hours. *Result negative.*

Case 9, J. W., m., 65 years. On 3. II. 17, at 4.30 p.m., 2.8 c.c. of filtrate (diluted 1 to 3) of blood from Case 4 taken on 2. II. (noon), 57 hours after the onset, were injected subcutaneously. The virus had then been outside the body for 28 hours.

The temperature was taken twice daily for seven days and thereafter four-hourly for 14 days. There was a rise to 99.4° F. on 7. II., and there were slight oscillations later not exceeding 99°, but insufficient to indicate infection. There were no symptoms, the patient feeling perfectly well during the whole period.

Remarks. Result negative. There were three other cases (8, 10, 11) inoculated with unfiltered preparations of blood taken at the same time, all of which were *positive*.

The case is fully discussed under the section dealing with filtrability.

Subcutaneous inoculation with 1.3 c.c. of washed corpuscles from Case 4. The blood was taken 57 hours after the onset, and was kept outside the body 28 hours. *Result positive.*

Case 10, P. M., m., 45 years. On 3. II. 17, at 4.30 p.m., 1.3 c.c. of washed corpuscles from Case 4 (*q.v.*) were injected subcutaneously into this case. The blood from which the corpuscles were derived had been taken from Case 4 on 2. II. 17, at noon, that is, 57 hours after the onset. The virus was kept outside the body 28 hours.

12. II. 17. At 5 p.m. he became suddenly ill with severe frontal headache and flushing. During this night he felt hot, flushed and headachy. The temperature rose steeply in the evening, reaching 101° F. at midnight.

13. II. Face flushed, eyes injected, tongue coated, no coryza. He stated that the headache had now practically gone. There was an erythematous flushing of the back but no rash. Temperature varied between 100° and 101° F. Blood was taken for experimental purposes. The Bordet-Gengou (compluetic) test was negative.

14. II. Face still flushed, eyes injected, tongue coated. He felt well. A faint mottling on the back. Temperature varied between 100° and 101° F.

Etiology of Dengue Fever

15. II. Temperature lower, the highest point reached being 100° F. There was still faint mottling on the back. The patient felt well. Another sample of blood was taken.

16. II. Maximum temperature 100.2° F. Mottling still visible on the back. The patient felt well.

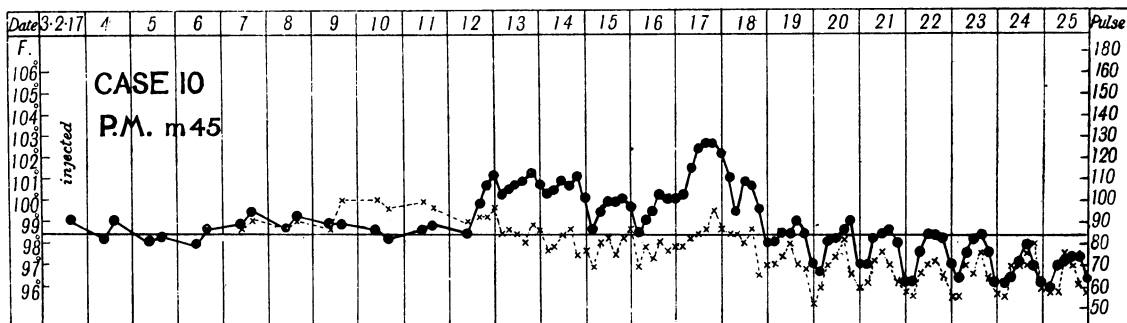
17. II. Rash still present on back and faintly marked on chest. He complained of feeling hot. Temperature rose to 102.8° F.

18-19. II. He did not feel so well. Temperature fell from 102° F. to subnormal.

20. II. He felt well. Maximum temperature 99° F. A third specimen of blood was taken.

After this the temperature did not rise above 99° F., and after 21. II. 17 was normal or subnormal. He was allowed up on 22. II. The rash was still present but he felt well.

Remarks. The inoculation undoubtedly caused a definite attack of dengue. The incubation period was nine days. The temperature variation



was definitely diphasic of the saddle back type. The pulse showed definitely the failure to respond proportionately to a rise in temperature so frequent in these cases. There was relative bradycardia from the beginning to the end of the period of pyrexia, and after the attack, the pulse was periodically absolutely slow. In this case we note again a rough correspondence in the chart between the oscillation of the pulse and that of the temperature, although the pulse is throughout the illness slow.

It will be noted that prior to the onset there was a period during which the pulse rate was higher than usual, but as soon as the pyrexia appeared the pulse became slower, only rising during the second part of the pyrexia to 95, although the temperature was 102.8° F.

Blood from this case, taken 22 hours and 67 hours after the onset, reproduced the disease, but blood taken eight days (ca. 190 hours) after the onset gave a negative result.

Experimental material. First specimen. Blood was withdrawn on 13. II. 17, at ca. 3 p.m., i.e. ca. 22 hours after the onset.

(a) 2 c.c. of this was squirted into about 8 c.c. of sterile 1.5 % sodium citrate in normal saline solution. This mixture was centrifuged free from corpuscles and filtered in the usual way after adding scrapings of an agar culture of *B. coli*. Filtration was rapid, ca. 5 c.c. of fluid being obtained in a few minutes. The dilution was 1 to 4.

Cultures were made by adding two loopsful of filtrate to 10 c.c. of broth and also by smearing a loopful on agar. These cultures remained sterile.

2.3 c.c. of the filtrate were used to inject Case 13 on 15. II. 17 at 4 p.m. with *positive result*.

(b) Serum from the clotted blood gave a negative Bordet-Gengou (compluetic) reaction.

Accidentally most of the clotted blood was thrown away, but there were a little serum and corpuscles left in the test-tube and these, after being shown to be sterile by culture, were injected into Case 14 on 15. II. 17 at 4 p.m. About 0.3 c.c. of blood was injected with *positive result*.

Second specimen. A large sample of blood, about 20 c.c., was withdrawn on 15. II. 17, at about midday, ca. 67 hours after the onset.

About 10 c.c. of serum were freed from corpuscles by centrifugalisation; scrapings of a culture of *Staphylococcus aureus* were added and the material filtered. Filtration was rapid. Cultures on broth (two loops) and agar (one loop) were sterile. 2 c.c. of the filtrate were used to inject Case 15 with a *positive result*.

Third specimen. Blood was taken on 20. II. 17, allowed to clot, and 1 c.c. of serum and corpuscles were used that day to inject Case 16, with *negative result*. This specimen was taken from Case 10 about 190 hours after the onset.

Subcutaneous injection of 2 c.c. of "washings" from Case 4, taken 57 hours after the onset. The material was kept outside the body for 28 hours.

Result positive.

Case 11, J. D., m., 74 years. On 3. II. 17, at 4.30 p.m., J. D. was injected subcutaneously with 2 c.c. of (1 to 5 diluted) corpuscle-free washings from the citrated blood of Case 4, the blood was taken on 2. II. at noon, 57 hours after the onset, the virus having been kept outside the body for 28 hours.

8. II. He complained of languor and some diarrhoea. Temperature normal.

9. II. He said he felt better but was "tired." Temperature normal.

10. II. Temperature rose to 99.0° F. at 4.25 p.m. During the night he felt so giddy and headachy that he was put to bed. (He was employed as night-watchman.) Temperature rose to 101.6° F. by midnight.

11. II. Temperature at 4 a.m. 102.4° F., ca. 101° to 102° during the day.

12. II. Face flushed, eyes and pharynx injected, tongue coated. There was pain in the neck and lumbar region and slight cough but no coryza. There was no rash. The patient complained of sleeplessness. Blood was taken on

this day and gave a *positive* result in the form of a filtrate in Case 17 and as "washings" in Case 18.

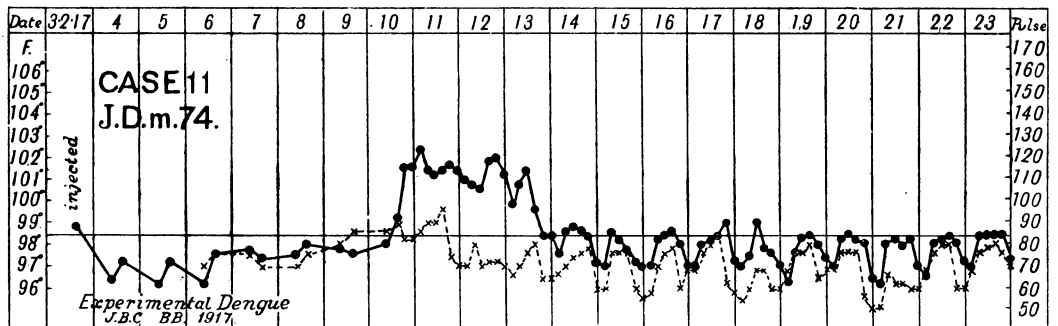
13. II. He felt much better and there were no pains. There was some suggestion of a rash. Temperature fell to normal.

14. II. A pinkish erythematous mottling over the whole back, chest, abdomen, thighs, shoulders and arms, but nothing on the legs, feet or hands. He felt well. Temperature about normal.

15. II. The rash fading. Blood taken on this date gave a *negative* result as a serum filtrate injected into Case 19. The temperature was normal at this time.

16-22. II. The rash faded gradually and the patient felt quite well. He was allowed up on 22. II. There were rises of temperature on 17 and 18. II. to 99° F.

26. II. He complained of weakness. The temperature had been normal or subnormal since 18. II.



Remarks. The incubation period, measured to the first rise of temperature, was seven days. The previous symptoms are regarded as premonitory. The chart shows a definitely maintained rise of temperature from 10 to 13. II. inclusive. On the evening of 13. II. the temperature fell sharply and thereafter, except for two isolated rises to 99° F. on 17 and 18. II., was normal or subnormal until 23. II. (end of chart).

The pulse-temperature relationship was "typical." Except for a slight increase on the day after the onset of fever, the pulse failed to respond to the rise of temperature. Relative bradycardia is marked. Towards the end of the chart are seen occasional periods of definite absolute bradycardia. Although the rash was not marked it was definite. *The result was positive.*

Experimental material. First specimen. On 12. II. at 3 p.m., 47 hours after the onset, about 10 c.c. of blood were taken from a vein. 7 c.c. of this were allowed to clot—about 1 c.c. of the serum was then abstracted for a Bordet-Gengou (compluetic) test (which proved negative), and also another 1 c.c. of serum and corpuscles was abstracted for injection purposes but not used. The remaining serum and clot (about 5 c.c.) were mixed with about

10 c.c. of a watery emulsion of colon bacilli and shaken well together. After allowing the solid material to deposit, the more fluid part of the mixture was again equally diluted with *tap-water* and filtered in the usual manner through a Pasteur-Chamberland F. filter.

About 5 c.c. of filtrate were rapidly obtained (the candle still retained about 6 c.c. of material). The filtrate was tested by making cultures on agar (one loop) and on broth (two loops), and these remained sterile. The specimen was labelled "blood filtrate, Case 11," and about 2 c.c. were used to inject Case 17 with a *positive result*. The dilution was approximately 1 to 5.

About 2 c.c. of the original blood was mixed with about 5 c.c. of 1.5 % sodium citrate normal saline solution. The mixture was centrifuged for half-an-hour and the supernatant fluid removed. Some haemolysis had occurred and the supernatant fluid still contained some red corpuscles. The supernatant fluid was recentrifuged, poured off, and left overnight. On the next day this was again recentrifuged and the supernatant fluid removed and labelled "washings from Case 11." 2.6 c.c. were used to inject Case 18 with a *positive result*.

We have no note as to the microscopical examination of the final fluid but it is safe to say it was then free from corpuscles. The dilution was approximately 1 to 2½.

Second specimen. About 15 c.c. of blood were taken on 15. II. 17 at about noon, and the serum was centrifuged free from corpuscles. About 3 c.c. of the serum, distinctly stained with haemoglobin, were obtained and this was diluted equally with water. To the dilute material a loopful of an agar culture of *Staphylococcus aureus* was added. It was filtered in the usual way through a Pasteur-Chamberland F. candle. The filtrate came through rapidly and was labelled "*serum filtrate from Case 11.*" Cultures made on agar (one loop) and broth (two loops) proved sterile. This filtrate was used to inject Case 19 with a *negative result*.

Subcutaneous injection of 0.3 c.c. (5 minims) of untreated blood from Case 4, ca. 130 hours after the onset. Virus kept outside the body less than 24 hours. *Result negative.*

Case 12, P. B., m., 61 years. On 6. II. 17, at 10 a.m., he received subcutaneously 0.3 c.c. of serum and corpuscles from Case 4, which had been taken on 5. II. about 130 hours after the onset. The virus was kept outside the body for less than 24 hours.

The temperature and pulse were taken twice daily for six days, and then every four hours for 16 days, but no indications of infection followed.

Remarks. The injection gave a *negative result*, this indicating that the virus was not present in the blood of Case 4 on the sixth day (ca. 130 hours) from the onset.

Subcutaneous injection of 2.3 c.c. of citrated plasma filtrate from Case 10.

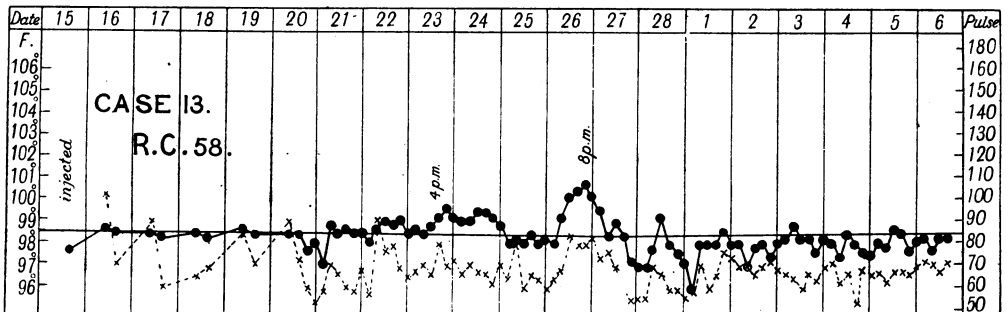
The blood was taken about 22 hours after the onset. Virus kept outside the body 59 hours. *Result positive (mild).*

Case 13, R. C., m., 58 years. On 15. II. 17, at 4 p.m., R. C. was injected subcutaneously with 2.3 c.c. of citrated plasma filtrate taken from Case 10. The blood was withdrawn from Case 10 at 3 p.m. on 13. II. or 22 hours after the onset. The virus was kept outside the body for 48 hours.

21-22. II. The temperature was as high as 98.8° F. at 8 a.m. on 21. II. but thereafter remained about normal until midday on 22. II., when it reached 99° F.

23. II. Temperature was 99.2° F. at 4 p.m., and 99.6° at 8 p.m. The patient complained of feeling "out of sorts," and was put to bed for observation.

24. II. Temperature remained at 99° F. during the day, rising to 99.4° in the evening. He had slight headache and depression and also a faint flushing of the face and back.



25. II. Temperature fell and the patient felt well.

26. II. Patient complained of headache during the previous night. Tongue coated, face and back flushed, but there was no rash. During the afternoon and evening, the temperature rose rapidly, reaching 100.8° F. at 8 p.m.

27. II. Temperature subnormal during the morning, and 99° F. at midday.

28. II. Since 28. II. the temperature had been normal. On this date the patient complained of pain in the chest and was not so well.

3. III. Slight headache and flushing of the back were present. Tongue coated.

There was nothing further to note after this date, and the patient got up on 5. III.

Remarks. It is hard to fix the time of onset of the pyrexia, as will be seen by reference to the chart. As early as 21. II. there was a rise to nearly 99° F. and during 22. II. the temperature was on the 99° level. On 23. II. the temperature rose definitely, reaching 99.6° F., and on 24. II. the highest point was 99.5° F. On the evening of 26. II. there was a steep rise to 100.8° F.

at 8 p.m., but by 8 a.m. on 27. II. this had subsided to rise to 99° at midday. Thereafter, with the exception of one slight rise to 99·2° on 28. II. the temperature was practically normal.

Considering the occurrence of symptoms, mild but definite, on the evening of 23. II., it is probable that the onset occurred about this time and was followed by the mild attack of dengue. Assuming that the evening of 23. II. represents the beginning of the attack, the incubation period was eight days. The type of chart was definitely diphasic with an initial slight pyrexia, and a secondary more definite pyrexia on 26. II.

The pulse is characteristic in both phases, failing almost completely to respond to the rise of temperature.

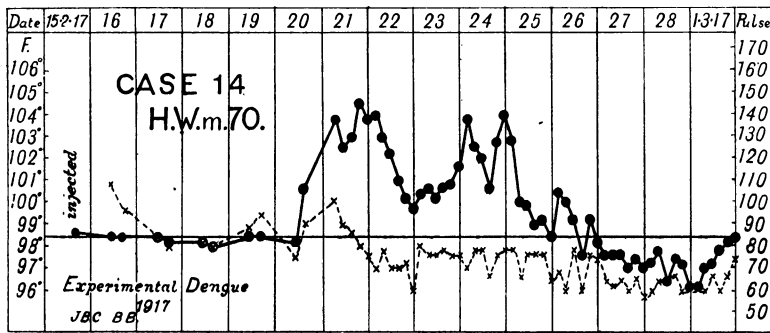
Experimental material. It was impossible to utilise the blood from this case for further experiments as there were no volunteers obtainable.

Subcutaneous injection of about 0·3 c.c. of untreated blood (serum and corpuscles) taken from Case 10, about 22 hours after the onset. Virus kept outside the body 49 hours. *Result positive.*

Case 14, H. W., m., 70 years. On 15. II. 17, at 4 p.m., H. W. was injected with about 0·3 c.c. of serum and corpuscles taken from Case 10 on 13. II. at 3 p.m., *i.e.* 22 hours after the onset. The virus was kept outside the body for 49 hours.

20. II. Temperature 100·6° F. at 3 p.m. Patient said he felt “flushed” but he had no other symptoms.

21. II. Temperature 103·8° F. in the morning, and 104·6° in the evening, but the patient said he felt well. Face flushed, eyes injected, tongue coated. A distinct erythematous flushing of the back but no definite rash.



22-23. II. A faint mottling on the back but nothing else to note. Patient felt well. A distinct fall of temperature occurred to 100° F. but it gradually rose again.

24. II. Patient said he did not feel so well. Temperature rose sharply to 103·8° F. in the early hours of the morning, and fell during the day to 100·6° F., rising again at night to 104°.

25. II. Temperature fell rapidly to 99° F. The patient felt well.

26. II. Temperature rose to 100.4° F., but fell again to subnormal, rising again at 8 p.m. to 99.2°.

27. II. Temperature subnormal and remained normal or subnormal afterwards until 2. III. Patient allowed up on 1. III.

Remarks. The incubation period is about five days. The chart is perhaps the most typical of the series. It shows a distinct diphasic variation with maximum points of 104° F. and over, and a distinct remittent period.

The pulse was absolutely characteristic. It rose slightly with the first rise of temperature but thereafter fell and remained normal during the subsequent pyrexia. The occurrence of a temperature of nearly 105° F. with a pulse rate of 80 needs no further comment.

The extraordinary absence of symptoms was most impressive and is a striking instance of a pathological process affecting the thermo-regulatory system without obviously affecting any other.

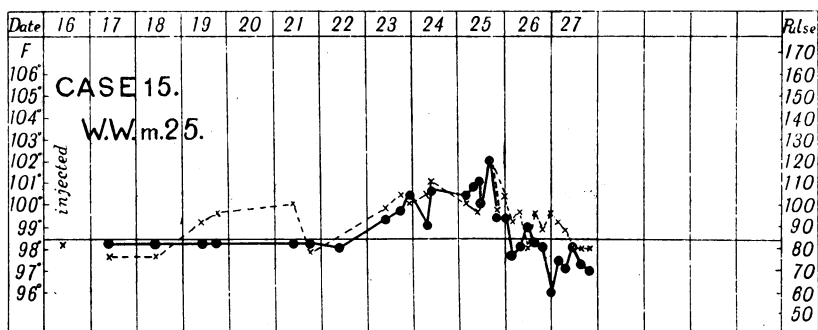
Experimental material. Although blood was twice taken with a view of using it for further experiments, the absence of volunteers prevented us making any use of the material.

Subcutaneous injection of 2 c.c. of undiluted serum filtrate from Case 10.

The blood was taken 67 hours after the onset. Virus kept outside the body 22 hours. *Result positive.*

Case 15, W. W., m., 25 years. On 16. II. 17, at 10 a.m., this case was injected subcutaneously with 2 c.c. of a "serum filtrate" from Case 10. The blood was taken from Case 10 on 15. II. at noon (67 hours after the onset). The temperature was normal until 23. II.

23. II. The patient became ill with typical and fairly severe symptoms. Malaise, bad headache and body pains were present. He continued to work. His temperature rose from 99.3° F. in the morning to 100.4° at 10 p.m.



24. II. Temperature at 7.30 a.m. 99° F. and at 9 a.m. 100.6°; it was not taken later that day. He was still going about his work but felt very sick. General pains were marked. There was pain in the eyes. He had a distinct

red mottled punctiform rash on the back, chest and buttocks, but not on the legs and arms.

25. II. Temperature rose to 102° F., but fell rapidly during the night. This day he remained in bed and felt very sick. Malaise and general pains were severe.

26. II. Maximum temperature 99° F.

27. II. Temperature was subnormal. He felt better and resumed ordinary duties. No further notes were taken but the rash was visible for several days and the patient complained of tiredness.

Remarks. The incubation period was seven days. This case was absolutely typical in symptoms and rash. The temperature variation is apparently monophasic. The pulse is not typical but it should be noted that this case had a tendency to a high pulse and was of a distinctly nervous temperament.

The blood from this case injected into Case 20 gave a *doubtful result*.

Experimental material. Blood was taken on 24. II. (24 hours after the onset), and 1.2 c.c. of the serum and corpuscles were injected into Case 20 on 27. II.

15 days later, Case 20 became febrile. His illness was most atypical, and cannot be considered definitely as dengue, especially as an attempt at further passage failed.

Subcutaneous injection of 1 c.c. of serum, containing corpuscles, taken from Case 10 on the eighth day of illness (about 190 hours after the onset).

The virus was kept outside the body at most a few hours. *Result negative.*

Case 16, A. A., m., ? age. On 20. II. 17, blood was taken from Case 10 and allowed to clot, and 1 c.c. of the serum was injected into this case at 5 p.m. The blood was taken eight days (190 hours) after the onset of Case 10. The virus was kept outside the body at most a few hours.

The chart was taken twice daily until 27. II., and thereafter every four hours until 13. III., and showed nothing to indicate any reaction to the injection.

Remarks. A *negative* result was obtained with serum from blood taken from Case 10 about 190 hours after the onset.

Subcutaneous injection of about 2 c.c. of blood filtrate from Case 11. The blood was taken 47 hours after the onset. Virus kept outside the body two days. *Result positive.*

Case 17, N. K., f., 28 years. On 14. II. 17, this patient received about 2 c.c. of a blood filtrate from Case 11 (*q.v.*). This had been prepared from blood drawn on 12. II. at 3 p.m. (47 hours after the onset of Case 11).

We have not a detailed history of this case as the circumstances of the volunteer did not permit us to take a chart, and when she became ill it was difficult to observe her frequently. The temperature was taken once daily

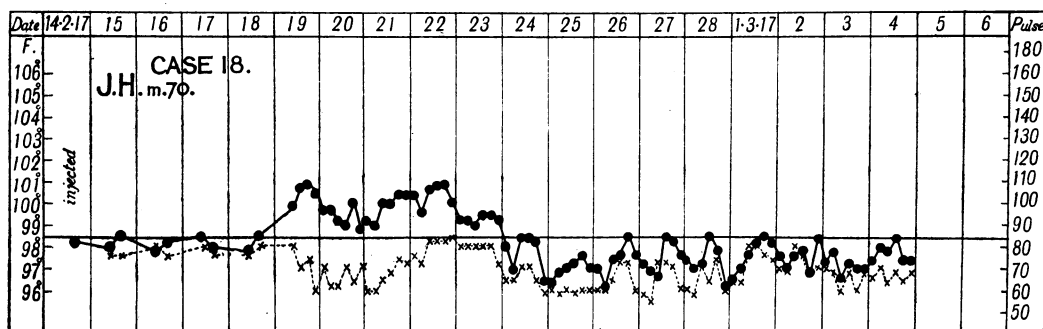
until 21. II. and was always normal and the pulse between 76 and 86. It was impossible to fix the onset as the patient did not say anything about feeling ill until we observed the rash, and then the history was not definite as to dates. But on 26. II. we observed that she had a very distinct rash, and on taking her temperature on the evening of that day it was found to be 100.3° F., with a pulse of 100, and the patient had the typical appearance of a dengue patient with pains, headache, lassitude, etc., and showed a bright typical rash on the face, forearms, chest and back (further examination was not made). The girl was obviously ill for some days after this, but refused to go to bed and was extremely anxious that her employers should not know that she had dengue, so further investigation was not pressed. There was no doubt that her case was very typical, the rash being one of the best of the series.

Remarks. Typical moderately severe dengue with well-marked rash.

Subcutaneous injection of 2.6 c.c. of "washings" from Case 11. The virus was taken from Case 11 47 hours after the onset, and was kept outside the body 48 hours.

Case 18, J. F. H., m., 70 years. On 14. II. 17, at 3 p.m., the case was injected subcutaneously with 2.6 c.c. of "washings" from Case 11 (*q.v.*). The blood was drawn from Case 11 on 12. II. at 3 p.m., 47 hours after the onset. The virus was kept outside the body 48 hours.

18. II. The patient complained of severe headache and pain in the chest on the afternoon of this date, but the temperature was normal.



19. II. At 10 a.m. the temperature was 99.8° F. and the pulse 80. Face flushed, eyes injected, tongue coated. There was severe headache. There was no rash but the back was erythematous. At 6 p.m. the temperature was 100.8° F. and the pulse 74. The patient was sleepless at night.

20. II. Headache still severe. Aspirin was given. There was no rash. The temperature was lower during the day (99.6° F.), but at 6 p.m. it rose to 100°, falling at midnight to 98.8°. He had a sleepless night.

21. II. There was nausea, pain in the chest, and headache requiring

aspirin. There was no rash. In the morning the temperature was 99° F., but in the evening rose to 100·4°.

22. II. Headache better. He had slept well during the night. Temperature reached 100·8° F., but fell during the night. Pulse for the first time rose somewhat, reaching 84 at 10 p.m.

23. II. He had still slight headache and anorexia, but felt better. Temperature not above 99·4° F. There was still no rash.

24. II. He complained of weakness and anorexia. The tongue was coated. Temperature normal or subnormal on this date and subsequently.

Remarks. The incubation period was between 4 and 5 days (about 4 days 19 hours to the first definite pyrexia). This is shorter than the usual incubation period. The temperature variation was diphasic with high points on 19 and 22. II.

The pulse was relatively slow throughout the pyrexial period. It was especially slow during the first phase of the fever.

Subcutaneous injection of 2·3 c.c. of dilute serum filtrate from Case 11. The blood was taken from Case 11, 115 hours after the onset, and was kept outside the body 70 hours. *Result negative.*

Case 19, T. B., m., 55 years. On 18. II. 17, at 10 a.m., Case 19 received subcutaneously 2·3 c.c. of a (1 to 1) diluted filtrate from the serum of Case 11 (*q.v.*). The blood was drawn from this case on 15. II. at about noon, about 115 hours after the onset. The virus, if present, would have been outside the body for 70 hours.

The temperature and pulse were taken twice daily for ten days, then every four hours for 13 days. The temperature reached 98·8° F. on 21. II., but thereafter was normal or subnormal.

Remarks. The late stage, at which the blood was taken from Case 11, by itself is enough to explain the *negative* result.

Subcutaneous injection of 1·2 c.c. of untreated blood from Case 15, taken 24 hours after the onset, and kept outside the body about 72 hours. *Result doubtful.*

Case 20, C. L., m., 70 years. On 27. II. 17, at 7 p.m., the case was injected subcutaneously with 1·2 c.c. of blood from Case 15, taken on 24. II. (about 24 hours after the onset). The virus was kept outside the body about 72 hours.

The temperature and pulse were taken twice daily for five days and then every four hours for three weeks. There was a rise to 99·2° F. on 5. III., about six days after the injection, but thereafter the temperature was practically normal. On 13. III. the temperature reached 99°, and on 14. III., 15 days after the injection, it rose to 100·2°, then fell rapidly, rose next day to 99·8°, fell again, and rose next day to 100°, falling rapidly again. The following day the maximum was 99°, and thereafter the chart was normal until 25. III., when the patient was discharged.

Symptoms. On 6. III. he developed a mild attack of herpes. On 7. III., although the temperature was only 98·8° F. at its maximum, there was some flushing of the face and back, and slight coating of the tongue. After this until 14. III. nothing was noted. Examined on this date the patient stated that he became shivery the previous evening at 7 p.m. He had some slight headache, some injection of the eyes, no coryza and slight cough without expectoration. On 15. III. there was an erythematous flushing of the back, no rash and a slight cough. On 16. III. he complained of nausea but otherwise remained well. On 17. III. he stated that he sweated every night. The tongue was slightly coated. Nothing else was noted after this.

There was relative bradycardia with the pyrexia on 14, 15 and 16. III., but no absolute bradycardia.

Remarks. The case cannot be definitely regarded as dengue, nor can it be stated that it was not.

In view of the failure to transmit infection from this case to Case 21, and the atypical nature and long incubation period, it is best to regard the case as *doubtful*.

Experimental material. Blood, taken from this case on 14. III., on the second (?) day of illness but the fifteenth after inoculation, failed to convey infection to Case 21.

Subcutaneous injection of 2 c.c. of untreated blood from Case 20, taken on the second (?) day of illness, and kept outside the body about 48 hours.

Result negative.

Case 21, H. McD., m., 42 years. On 16. III. 17, at 4.30 p.m., this patient was injected with 2 c.c. of blood taken on 14. III. from Case 20. He had thereafter no symptoms of dengue and no pyrexia, although observed daily for several weeks.

Subcutaneous injection of 2 c.c. of serum and corpuscles from a guinea-pig injected 7½ days before with blood from Case 10, taken 22 hours after the onset.

Case 22, P. W. P., m., 71 years. The details of this case are found under Part III, Animal Experiments (bottom of p. 233).

REFERENCES.

- (1) CLELAND, BRADLEY and MACDONALD (2. IX. 1916). On the Transmission of Australian Dengue by the Mosquito *Stegomyia fasciata*. *Med. Journ. of Australia*.
- (2) ——— (5. II. 1918). Dengue Fever in Australia. *Journ. of Hyg.* xvi. 317-418, 9 charts; also in *Seventh Report of the Microbiological Laboratory of the Dept. of Public Health, N.S.W.*
- (3) GRAHAM (1. VII. 1903). The Dengue; A Study of its Pathology and Mode of Propagation. *Journ. of Trop. Med.* vi. p. 209.
- (4) ASHBURN and CRAIG (1907). Experimental investigations regarding the Aetiology of Dengue Fever. *Philipp. Journ. of Sc.* II. 93.
- (5) BANCROFT (I. 1906). On the Aetiology of Dengue Fever. *Aust. Med. Gaz.* p. 17.