OBSERVATIONS ON THE WEIL-FELIX REACTION IN TSUTSUGAMUSHI DISEASE[1].

By J. W. WOLFF.

(*From the Pathological Laboratory, Medan-Sumatra.*)

(With 3 Charts.)

Introduction.

Cases of a disease of the typhus group, with symptoms very similar to those of the tsutsugamushi disease in Japan, have been observed in certain areas of the east coast of Sumatra ever since Schüffner in 1909 published his first observations on this disease (then called pseudo-typhoid). Although not exactly alike in epidemiology and clinical features (see Schüffner, 1909; Maasland, 1926; Kawamura, 1930), it is generally admitted that these two mite-borne diseases belong to the same group.

In 1925 Fletcher and Lesslar published several observations on another typhus-like fever, "tropical typhus," the clinical symptoms of which were much like those of tsutsugamushi, type Schüffner, but differed mainly in two ways; firstly as to the primary ulcer and bubo, which were always absent in their tropical typhus cases, while secondly there was no evidence that a mite was the vector as is the case with the tsutsugamushi disease. Fletcher and his co-workers stated further that two types of tropical typhus occurred with slight variation in the course of the fever, the main difference being the Weil-Felix reaction. In the urban type, "shop" typhus (Fletcher, 1930), agglutinins were formed in the patient's serum against the ordinary *Proteus* X 19 strain, type "Warsaw." In the rural type, "scrub" typhus, a positive agglutination reaction occurred with the non-indologenic variety called the "Kingsbury" strain. In all these latter cases the agglutination of the patient's serum with the ordinary *B. proteus* X 19 strain invariably gave a negative result.

The observations of Fletcher and his co-workers were soon confirmed in other localities. In Sumatra I was able to observe several cases of tropical typhus of both types (1929). Since cases of scrub typhus occurred side by side with cases of tsutsugamushi, it was of importance to see whether a positive Weil-Felix reaction would occur during the course of tsutsugamushi disease and whether or not it would assist in the differential diagnosis of the two diseases.

[1] A short note on these observations will be published in the *Geneeskundig Tijdschrift voor Nederlandsch Indië.*

REVIEW OF EARLIER LITERATURE.

In cases of tsutsugamushi disease in Japan, the Weil-Felix reaction is stated to be negative or of so low a titre as to have no diagnostic value (Ishiwara and Ogata, 1923; Kawamura, 1926, 1930). Apparently these authors have used the customary indologenic strain of X 19. In a recent publication, Fletcher, Lesslar and Lewthwaite (1929), discussing the differential diagnosis between scrub typhus and tsutsugamushi disease in the Federated Malay States, state that, "...the agglutination reaction with the non-indologenic strain of *B. proteus*, known as the Kingsbury strain, also serves to separate scrub-typhus from the tsutsugamushi disease, because the titre of agglutination is far higher in the former than in the latter; at the same time this test shows the close relationship of the two fevers, because tsutsugamushi is the only disease, except scrub-typhus, in which the blood has been found to agglutinate the Kingsbury strain in dilutions higher than 1 in 200...." The highest titre Fletcher found in three out of seven cases was stated to be 1 in 240.

THE AUTHOR'S OBSERVATIONS.

The present study deals with observations on forty-five cases of tsutsugamushi, type Schüffner. Since 1926, agglutinations with suspensions of the "Warsaw" and "Kingsbury" strains have been performed with most of the samples of human sera sent in, for diagnostic purposes (Widal tests, etc.), to the Pathologisch Laboratorium in Medan. The sera were sent in from different cases of fever, amongst which there have been every year several well-established cases of tsutsugamushi disease. However, since nearly all these sera were sent in during the first days of the disease, those agglutinations invariably have had a negative result. And as soon as the primary ulcer was found, for diagnostic purposes it was not necessary to send in serum for agglutination tests during a later phase of the disease.

In order to investigate this matter further, a series of cases of tsutsugamushi disease have been examined as far as possible every few days. All these cases were diagnosed and treated by Dr M. Straub of the Bangkattan Hospital of the Deli Maatschappij. I am very much indebted to him for sending me sera of his patients on several occasions, and for allowing me to make use of his case records.

From the beginning of 1929 up to March 1930 sera from fifty tsutsugamushi cases have been received. In this communication I have not made use of the notes of five cases, since in four of these it was thought afterwards that the occurrence of a primary ulcer had not been established with certainty. The fifth case had a primary ulcer, but showed, after a series of totally negative agglutination tests, a positive agglutination on the fifteenth day of the disease with the "Warsaw" strain up to 1 in 1000, while on the seventeenth and nineteenth days again a negative agglutination was noted. As all the other positive

agglutinations have occurred with the "Kingsbury" strain, this result may probably have been misrecorded.

The other forty-five cases all showed a typical primary ulcer and nearly all of them a corresponding bubo near the site of the ulcer. The number of agglutination tests performed in these cases is set out in Table I.

Table I.

No. of tests performed in each case	Cases of tsutsugamushi where the Weil-Felix test with the "Kingsbury" strain	
	Became positive	Remained negative
1	2	2
2	3	1
3	3	4
4	9	2
5	6	4
6	3	1
7	1	.
8	3	.
11	.	1
	30	15

It will be seen that only in thirty out of forty-five cases was the agglutination positive with the "Kingsbury" strain during some phase of the disease. In fifteen cases the agglutination was negative. It might naturally be presumed, that in those cases, where only one or two tests were performed during the course of the disease, an agglutination made during a later phase might have been positive. However, some observations, which are discussed later on, show very clearly that in some cases no agglutinins could be detected during the whole course of the disease.

The following technique of agglutination tests has been employed. Saline suspensions of the motile flagellated "Warsaw" and "Kingsbury" strains were used after growth for 24 hours on sloped agar. The bacillary suspensions were killed by 1 per cent. formalin. One-half of each serum sample was heated for an hour at 56° C. and with the heated and unheated serum samples agglutination tests were performed in dilutions up to 1 in 1000. The object of heating a part of the serum was to see whether there was any difference in titre between the unheated and the heated serum portion. According to Prausnitz (1920), the specific agglutinins in the serum of typhus patients are for the greater part destroyed by heating the serum, while the *proteus*-agglutinins, due to infection with some ordinary *B. proteus vulgaris*, are heat stable[1]. This difference is due to the different resistance to heat of O and H agglutinins (Weil and Felix, 1917; Felix and Olitzki, 1929). For their differentiation in routine diagnosis it is recommended by Felix (1930) to use side by side suspensions of O and H variants of *B. proteus* X.

[1] In my previously quoted article on tropical typhus, some agglutination records show a very marked difference in titre between the unheated and heated serum tests.

Table II. *Positive Weil-Felix reactions ("Kingsbury" strain) in thirty cases of tsutsugamushi disease.*

Year	No.	Day of disease	Serum Un-heated	Serum Heated
1929	675	6	100	.
		13	100	.
		18	—	.
	891	12	100	.
		17	100	.
	1,872	7	—	.
		12	—	.
		19	1000	250
	1,923	8	1000	1000
	2,351	8	—	.
		15	—	—
		20	1000	500
		25	100	50
	2,396	16	250	50
	4,236	14	1000	1000
		23	1000	500
		31	25	—
	5,769	5	100	50
		7	250	250
		11	250	100
		13	250	100
		15	100	25
		17	50	25
	6,395	5	—	.
		13	250	.
		15	500	.
		18	50	.
		20	—	.
	9,509	6	—	—
		19	100	50
		21	—	—
		23	—	—
		25	—	—
	13,244	4	—	.
		11	—	—
		16	100	50
		18	1000	100
		20	1000	250
		22	1000	100
		24	1000	100
		26	500	50
	14,120	11	1000	1000
		13	1000	500
		15	1000	500
		17	500	50
1930	640	6	100	.
		8	1000	.
		10	1000	.
		12	1000	.
	641	4	—	—
		6	—	—
		8	—	—
		10	—	—
		12	500	100
		14	1000	500
		16	1000	1000
		18	1000	1000
	732	6	—	—
		7	—	—
		9	50	—
		11	500	.
		13	1000	.
		15	1000	.
1930	702	6	—	.
		11	25	.
		13	50	.
		16	50	.
		18	25	.
		20	25	.
		22	25	.
		28	—	.
	905	6	—	—
		10	50	250
		12	500	250
		14	500	100
		16	250	100
		18	100	50
	1,061	7	—	.
		11	—	.
		13	50	—
		15	50	25
		17	25	—
	1,179	12	—	—
		14	500	100
	1,382	11	50	25
		13	250	100
	1,644	15	250	.
		17	1000	.
		19	500	.
		21	500	.
	1,741	15	1000	250
		18	1000	500
		20	1000	1000
		27	100	50
	2,201	8	—	—
		18	500	50
		20	250	—
		22	50	—
		26	25	25
	2,248	10	—	—
		16	1000	1000
		17	1000	1000
		19	1000	250
	2,249	6	—	—
		11	25	—
		14	1000	500
		16	—	—
		18	—	—
	2,470	4	—	—
		9	—	—
		11	250	100
		13	—	—
	2,499	7	—	—
		9	25	—
		11	100	—
		13	500	250
		15	250	25
		17	100	—
		19	—	—
	2,507	9	25	—
		11	100	—
		13	250	—
		17	250	—
	2,600	5	—	.* —
		12	250	. 250
		21	500	250 250
		28	—	— —
	3,789	5	—	.* .
		12	100	. —
		14	250	100 100
		16	50	25 25
		18	50	— —

* Alcoholic suspension.

The titre recorded is the highest dilution in which the agglutination was visible to the naked eye. Except in the last two cases, where alcoholic suspensions also were used, all agglutinations were performed with formalin suspensions. Dilutions higher than 1 in 1000 were not tested. — signifies negative. . signifies not done.

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CASES WITH POSITIVE AGGLUTINATION TESTS.

Table II shows the agglutination results of all those cases where a positive agglutination occurred during the course of the disease. The agglutination tests with both the heated and unheated sera and the "Warsaw" strain, which were invariably negative, are not recorded in this table. In the last two cases of Table II agglutinations were also performed with the unheated serum and alcoholic suspensions of the two strains, following the technique of Bien and Sonntag (1917). It will be seen that with these alcoholic (O) suspensions, the titre was slightly lower than that reached when formalin suspensions were used.

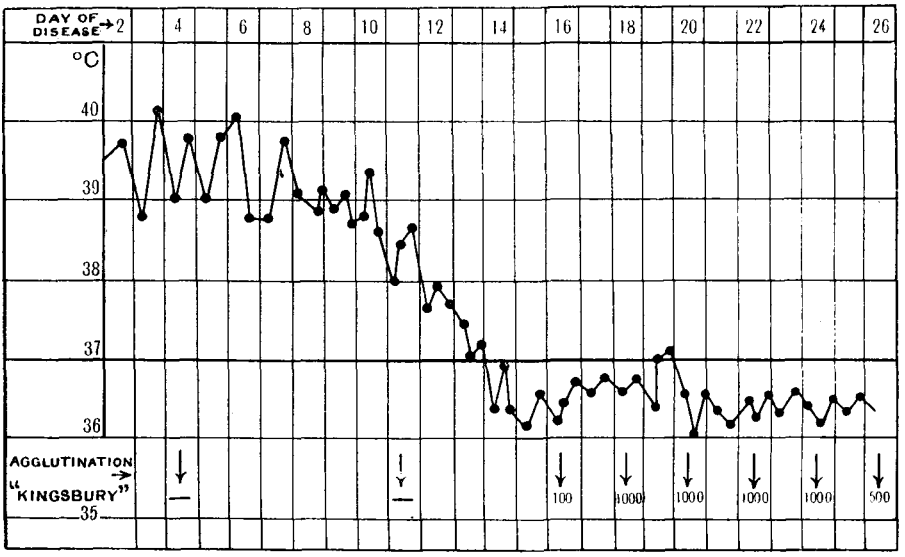

Chart I. No. 13,244. Javanese labourer, ± 30 years. In hospital Dec. 2nd–26th, 1929. On admission a slight patchy rash is noticed, spread over face, breast and back. In the right fossa inguinalis a tsutsugamushi ulcer is present, also a painful swollen bubo. Heart and lungs normal, spleen swollen. Blood count. 5700 leucocytes. Dec. 4th. Widal: typhoid 1/50. Cultures of blood, faeces and urine negative for *B. typhosus*. Urine trace albumen, diazo positive, urobiline positive. Dec. 7th. Rash faintly visible. Dec. 11th. Ulcer healed. Widal: typhoid 1/25. Blood, faeces and urine culture negative for *B. typhosus*. For Weil-Felix reaction see chart.

It will be seen from Table II that, in some cases, only one or two agglutination tests could be performed. In these cases it is difficult to prove that the recorded positive reaction is due to the tsutsugamushi infection and is not the result of an earlier infection with the virus of tropical typhus. But in twenty-four cases there is a definite rise in the titre during the course of the disease. Chart I, accompanied by short notes of case No. 13,244, serves as an example.

During the course of the fever no agglutinins had been formed on the fourth nor on the eleventh day of illness. On the sixteenth day an agglutination titre of 1 in 100 was noted in the unheated serum, which rose to 1 in 1000 on the eighteenth day and persisted till the twenty-fifth day. It might even have

been higher during that interval, but unfortunately higher dilutions were not used. The last test in this case was made on the day of the patient's dismissal, when the titre was found to have dropped to 1 in 500.

Another interesting case was No. 641. (See Chart II.)

This patient was first admitted with a fever during the course of which a well-marked positive Weil-Felix reaction with the "Kingsbury" strain developed. Since the titre of agglutination showed a rise on two following occasions, while the most careful inspection revealed no trace of an ulcer or

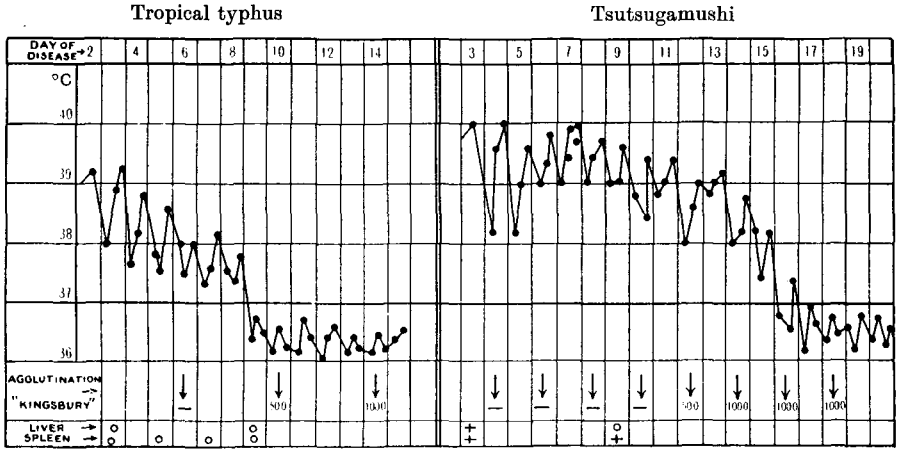

Chart II. No. 641. Javanese labourer, ± 24 years. In hospital June 7th–29th, 1929, and Dec. 30th, 1929–Jan. 21st, 1930. On admission patient complains of fever, headache. Heart normal, slight bronchitis. In urine no albumen, diazo, and trace urobiline. In blood malaria negative. 10,800 leucocytes. Cultures of blood, faeces and urine negative for *B. typhosus*. Widal on June 11th, typhoid 1/50, para-A 1/250 (patient had been vaccinated with T.A.B. in April, 1929). For course of fever and Weil-Felix reaction see chart. Six months later the same man is re-admitted with high fever. On the right side of the scrotum a typical ulcer with bubo. Spleen swollen, heart and lungs normal. Widal twice negative. In urine trace albumen, diazo and urobiline reaction both positive. On Jan. 8th a parotitis developed which healed in a few days. See chart and text for course of fever and Weil-Felix reaction.

bubo, the diagnosis tropical typhus (type "Kingsbury") was made. Six months later, the same man was readmitted to the hospital with high fever, showing a typical tsutsugamushi ulcer on the scrotum. Both times the man had worked in an area of newly cut secondary growth, where more cases of tsutsugamushi and tropical typhus occurred side by side. Till the twelfth day of his illness, the serum of this patient showed no trace of agglutinins for the "Kingsbury" strain; they had disappeared during the interval of six months. Thereafter, a strong agglutination up to 1 in 1000 was noted, still present at the time of his dismissal[1].

[1] As far as I know, this is the first evidence of the occurrence of these two diseases of the typhus group in the same individual after an interval as short as six months. In this case, the infection with the first virus (whether tropical typhus or tsutsugamushi without a primary ulcer) gave no lasting immunity against a later infection with the tsutsugamushi virus. Similar observations have been published from Japan (Kawamura, 1930).

CASES WITH NEGATIVE AGGLUTINATION TESTS.

As stated previously (see Table I), in fifteen out of forty-five patients suffering from tsutsugamushi disease the serum yielded negative reactions with the "Kingsbury" strain. In some of these cases the test has not been applied often enough to allow any definite conclusion to be drawn from the negative results recorded. But in other cases agglutinations were performed at a stage of the disease when a positive agglutination might have been expected, judging from those cases where the formation of agglutinins did take place. For comparison all positive and negative results, according to the day of the disease, have been recorded in Table III, while Chart III relates to a case with a negative Weil-Felix reaction throughout the course of the disease.

Table III. *Agglutination tests with the "Kingsbury" strain according to day of disease.*

Day of disease	Titre of agglutination							Total positive
	Negative	1/25	1/50	1/100	1/250	1/500	1/1000	
3	1	.	.	.	.	.	.	.
4	3	.	.	.	.	.	.	.
5	7	.	.	1	.	.	.	1
6	9	.	.	2	.	.	.	2
7	8	.	.	.	1	.	.	1
8	4	.	.	.	.	.	2	2
9	2	2	1	.	.	.	.	3
10	6	.	1	.	.	.	1	2
11	9	2	1	2	2	1	1	9
12	3	.	.	2	1	2	1	6
13	6	.	2	1	4	1	2	10
14	5	.	.	.	1	2	3	6
15	4	.	1	1	2	1	3	8
16	4	.	2	1	2	.	2	7
17	4	1	1	2	1	1	2	8
18	7	1	2	1	.	1	3	8
19	6	.	.	1	.	1	2	4
20	5	1	.	.	1	.	3	5
21	2	.	.	.	.	2	.	2
22	2	1	1	.	.	.	.	2
23	6	.	.	.	.	.	2	2
24	3	.	.	.	.	.	1	1
25	1	.	.	1	.	.	.	1
26	.	1	.	.	.	1	.	1
27	.	1	.	1	.	.	.	2
28	2	.	.	.	.	.	.	.
29	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.
31	.	1	.	.	.	.	.	1
	109	11	12	16	15	13	28	94

It will be seen that this case was clinically a typical tsutsugamushi. Instances of failure to form agglutinins have been recorded in classical typhus fever. There the disease either ran an extremely mild course or the case was a very toxic one, nearly invariably ending fatally, where we may presume, that the body failed to react with the formation of antibodies (for references see Felix, 1930). In our cases the reason for failure of production of antibodies is not known. All that can be said is that these cases with negative agglutination reactions were neither extremely mild nor very toxic, the mortality being nil;

the fever in two of these cases lasted for 22 and 26 days respectively, while in the others the duration of the fever was between 7 and 15 days.

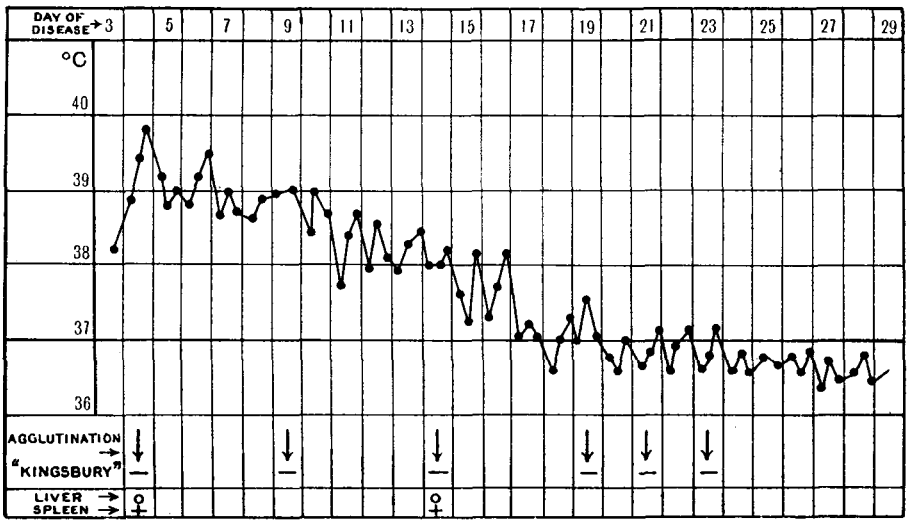

Chart III. No. 5061. Javanese labourer, ± 28 years. In hospital May 17th–June 24th, 1929. On admission fever, headache. Tsutsugamushi ulcer on left fossa inguinalis on the side of the scrotum with swollen inguinal glands. Widal negative. 5800 leucocytes. Cultures of blood, urine and faeces negative for *B. typhosus*. See chart for course of fever and Weil-Felix reaction.

DISCUSSION.

It has been shown that in thirty out of forty-five cases of tsutsugamushi disease a positive Weil-Felix reaction with the "Kingsbury" strain has been recorded. In many of these cases the agglutination reached a titre of 1 in 1000. The titre might even have been higher if further dilutions of the sera had been tested or suspensions of the O variants of the bacilli (Felix, 1930) had been used.

These facts support only the second statement quoted from Fletcher, namely, the close relationship between tsutsugamushi disease and scrub typhus. If further observations should reveal titres higher than 1 in 1000 occurring in cases of tsutsugamushi disease, the height of the titre will not form a useful method of differentiating these diseases. Perhaps the same objection might apply to the rather minute differences in the clinical course of these two diseases which are given by Fletcher and his co-workers as means of differential diagnosis. In all epidemics of infectious diseases fluctuations in severity of clinical symptoms occur and have been recorded of the same order as those observed in these diseases. Similar fluctuations are also to be seen in the case records published by Schüffner and Maasland[1]. In the present

[1] Even a primary ulcer was found only in a certain percentage of cases in the earlier descriptions of tsutsugamushi disease in Sumatra (78 per cent. out of 197 cases during 1915–21 and only in 59 out of the 156 cases described by Maasland). At that time Schüffner, having seen the primary ulcer in all his European cases, thought that its absence in some cases amongst native patients might be due to the fact that the ulcer was healed at the time of admission to the hospital. From the evidence provided by the more recent studies on tropical typhus it seems quite possible that some of the cases without ulcer might have been cases of so-called scrub typhus, that is if we regard the detection of a primary ulcer and bubo as the only valuable method of differentiation between tsutsugamushi disease and scrub typhus.

state of our knowledge of scrub typhus and tsutsugamushi disease it appears to me to be still an open question whether the two diseases are caused by different viruses or whether the same virus is involved in both diseases, but is spread by different vectors, of which the one does give a skin ulcer, and the other does not. In the case of scrub typhus, recent studies by Anigstein (1930) seem to make it possible to cultivate from the blood of patients strains of rickettsia-like organisms and to perform successful transmissions in guinea-pigs. Further experiments, combined with observations on immunity reactions in both diseases, may throw light on this question. In the meantime, for practical reasons, it seems advisable to separate tsutsugamushi disease from scrub typhus according to the presence or absence of the primary ulcer.

SUMMARY.

1. The Weil-Felix reaction in a series of forty-five cases of tsutsugamushi disease (type Schüffner) is recorded.

2. The agglutination reaction was negative in fifteen cases, the other thirty showing a positive reaction with the "Kingsbury" strain of *B. proteus* X.

3. The Weil-Felix reaction is not regarded as an adequate method for differentiating tsutsugamushi disease from scrub typhus, since high titres (1 in 1000) occur in a considerable proportion of cases of tsutsugamushi disease.

4. In the present state of our knowledge of the two diseases it is recommended that they should be separated according to the presence or absence of a primary ulcer and bubo.

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