tested. The Weil-Felix reaction is no exception to this rule, and it is of importance to know within what limits normal sera may react with *B. proteus X* 19. To arrive at a tentative conclusion on this point a series of 100 normal sera, mostly from lice-infested coolies, were examined with the following results:—All failed to shew the least trace of agglutination in a dilution of 1:16; 8 per cent. gave a feeble reaction in 1:8; 33 per cent. reacted in 1:4, and 59 per cent. failed to react at all in 1:4.

Conclusions.

The Weil-Felix reaction in Indians is of value (a) in confirming a diagnosis arrived at by clinical observation, (b) in establishing a diagnosis in typical cases in which the clinical evidence of typhus is doubtful, and (c) in arriving at a retrospective diagnosis in cases which have recovered, provided the interval since recovery is not too great.

My experience with the reaction in the Simla outbreak suggests that, employing Garrow's technique in the manner described above, a positive reaction in a serum-dilution of 1:32 and upwards is, for practical purposes, diagnostic of typhus fever. Further observations in other outbreaks and by other methods will be necessary before a definite standard can be arrived at for use in Indian epidemics of typhus.

ON A PSEUDO-ORGANISM IN THE BLOOD IN DENGUE.

By R. KNOWLES,

MAJOR, I.M.S.,

and

S. A. S. BIRAJ MOHAN DAS GUPTA, L.M.P.,

From the Protozoology Department, Calcutta School of Tropical Medicine.

Introduction.—The view that dengue is a spirochætal disease is one which has been entertained by many workers, although the arguments upon which such a theory rests appear to the writers to be but slender. Owing to its rather distant resemblances to yellow fever, to icterohæmorrhagic jaundice and to the relapsing fevers; to its proved *Aëdes* (*Stegomyia*) transmission (Cleland, Bradley and McDonald, 1916; and Chandler and Rice, 1923) and possible *Culex* transmission (Graham, 1903; Craig, 1907); to the very considerable volume of experimental work upon the virus of dengue attended with negative results,—which might be compatible with the presence of a leptospira in the blood in very scanty numbers only; a view appears to be prevalent that the causative micro-organism of dengue is probably a spirochæte.

The positive findings in favour of such a view up to the present date are as follows:—

(1) Couvy (1921), in the 1920 epidemic in Beyrout saw five or six times very scanty spirochætes in the blood on dark ground examination of dengue cases one to two hours *before* the onset of fever. He described the organism as an extremely thin spirochæte with tapering ends and with only 2 to 3 turns.

(2) Couvy (1922), in a second epidemic in 1921 in Beyrout again found the same spirochæte in the blood of dengue cases, not only before the onset of fever as in 1920, but at from 3 to 48 hours after the febrile onset. The blood of dengue cases was inoculated into rabbits; fever of a dengue type followed with the same spirochætes present in the animals' blood during both the primary fever and the secondary rise of temperature. The spirochæte was passaged from rabbit to rabbit. In some areas neither *aëdes* nor culex mosquitoes were present, and his experiments incriminate *Phlebotomus pappatasii* as the insect carrier in connection with the epidemic.

(3) Lieut.-Colonel J. W. D. Megaw, I.M.S., in 1921 found on dark ground examination of blood taken from a typical case of dengue during the first few hours of the disease one single form representing a leptospira. This observation was not published, as it was a solitary finding only, but was mentioned in a footnote to his 1923 paper (Megaw, 1923). Thanks to Colonel Megaw's kindness we are here able to reproduce a sketch taken at the time of the appearances presented by this organism (figure 1).

(4) If we accept Colonel Megaw's views, (Megaw, 1923)—that sandfly fever and dengue are essentially fevers of the same clinical type and should be classified as sandfly-dengue and mosquito-dengue respectively, then we may here include the finding by Couvy (1921) of a spirochæte in the blood of two cases of sandfly fever at 3 and at 24 hours after the onset of the fever, with successful passage of the virus to a rabbit who contracted fever and shewed spirochætes in its blood at the time of onset of the fever. Also the findings by Whittingham (1922) on sandfly fever in Malta. Spirochætes were found on dark ground examination of direct blood cultures in 6 out of 26 cases, and three primary strains were established in sub-culture.

We are a little doubtful, however, to what extent the view that dengue and sandfly fever are the same disease can be accepted, and it is to be noted that Whittingham's organism differed entirely from Couvy's spirochæte of dengue, the former "being morphologically indistinguishable from *Leptospira icterohæmorrhagiæ*, the average length being 10 to 15 μ."

(5) Vervoort (1922), during an epidemic in the Dutch East Indies of an acute fever of benign type associated with rheumatoid and vaso-motor symptoms and occasionally with a terminal rash and jaundice, isolated in five cases a spirochæte resembling *L. icterohæmorrhagiae* and *L. hebdomadis* in blood films, in cultures, and once in an inoculated guinea-pig. Guinea-pigs proved very susceptible to the infection. Whether Vervoort was dealing with dengue or not we cannot say,

but the symptoms described are suggestive of dengue.

(6) van de Velde (1923), working in Sumatra, found a leptospira resembling *L. hebdomadis* in the blood of 23 out of 430 cases of an unclassified fever. The author notes, however, that only two of the cases shewed symptoms suggestive of dengue.

The experimental evidence that dengue is a spirochætal disease therefore rests chiefly upon Couvy's findings. On the other hand the experimental evidence *against* the spirochætal hypothesis is very strong. Apart from the large volume of earlier work with completely negative results, one may instance the work of Koizumi, Yamaguchi and Tonomura (1917) in the 1915 epidemic in Formosa. These workers proved the infectivity of the blood in even as small an amount as 0.00005 mil. but failed to find any micro-organism. Finally Chandler and Rice (1923), working in a Texas epidemic, "made every effort to find such an organism (leptospira) if it were present." They examined 250 preparations from 70 cases by dark ground examination and numerous stained slides, but found nothing. In a few cases the urine was centrifuged and examined, but with negative results. Strenuous efforts to discover a leptospira by cultural methods and all attempts to infect experimental animals were with negative results. They proved transmission by *Aëdes (Stegomyia) ægypti*, but failed entirely with *Culex quinquefasciatus*. The incubation period of the disease after the bite of an infected mosquito varied from 4 to 6½ days. This most carefully controlled and thorough enquiry led these workers to the conclusion that there is no real evidence that dengue is a spirochætal disease and that its analogies with yellow fever have been over-emphasised.

Under these circumstances, and in view of the entirely contradictory character of the findings and views prevalent, we determined during the 1923 epidemic of dengue in Calcutta to lay aside all other work as far as possible for the time being, and to seize upon this opportunity to investigate the facts. Our results may be of some interest, as for a period of several weeks we were almost assured that we had isolated a true leptospira as the micro-organism of dengue, but the final and cumulative weight of the experimental findings, and especially those in the controls, force us to the conclusion that the forms observed were artifacts of a new and puzzling character. We may add that Chandler and Rice's paper did not come to hand until nearly the conclusion of our enquiry, which lasted from July the 25th to September the 30th, 1923.

The Calcutta Epidemic of 1923.—The dengue epidemic of 1923 in Calcutta was one of the worst that the city has ever experienced. From July to the end of September, Calcutta was almost prostrated by the disease. Probably some 40 per cent. of its inhabitants suffered from one or more attacks of dengue during this period. Thus at the University Students' Hostel, out of 260 students in residence no less than 165 went down with dengue. The *economic importance* of dengue is very considerable. Every case contracted means the loss of one week of working time by the individual, to say nothing of the after-effects and impaired working efficiency; and the epidemic must have cost Calcutta city, and its mercantile community especially, some lakhs of rupees in time and labour lost.

It would appear, further, that the Calcutta epidemic was but part of a more widespread pandemic. Bombay suffered severely in March to May, 1923; Lucknow and Delhi were suffering severely in September; the whole countryside around Calcutta within a radius of 40 miles was affected, and isolated cases—mostly among new arrivals from Calcutta—were reported in such hill stations as Ranchi, Shillong and Darjeeling.

The clinical aspects of the epidemic it is for other writers to deal with. The fever usually lasted for 3 or 4 days only without a secondary rise; but such secondary rise about the (5th or 6th ?) 7th day was seen in some 20 per cent. of the cases. The rash, coming out on the 4th day as the temperature fell, was well seen in many cases and was even followed by superficial desquamation. The joint pains were intolerable in some cases and tended to persist, together with some swelling of the ankles, for weeks together in convalescence. What was especially noticeable was the special tendency to relapses (? re-infections) in the same individual. Thus person after person would suffer from two or even three attacks of dengue during the three months, and the second or third attack might prove more severe than the first. Of immunity acquired during the actual dengue season itself there was but little clinical evidence. On the other hand certain individuals who had contracted dengue in previous years escaped scot free; and it would appear that the immunity-response to dengue is but slowly acquired over a period of some months, but that when acquired it may be very real. Possibly the virus of dengue may pass out of the blood stream at a very early stage of the disease into the connective and ligamentous tissues, to remain there for a period of some weeks or months, slowly dying out, responsible for the after-pains, but also acting as a slow and cumulative antigen. Any such hypothesis, however, must await proof or disproof until the causative organism has been discovered.

We had no lack of clinical material for the enquiry; indeed the enquiry itself was hampered by attacks of dengue among the laboratory personnel. In the Carmichael Hospital for Tropical Diseases some 30 per cent. of general in-patients contracted dengue in addition to their primary disease. Day after day kala-azar cases who had had a normal temperature for weeks and were well on the way to cure would suddenly go down with dengue and look pictures of misery. Two malaria patients,—one a benign tertian and the other a quartan,

as proved by blood examination—contracted dengue within 20 days of their last malarial rigor, although at the time on full cinchona treatment. In a third instance a laboratory worker at the School contracted a severe attack of dengue, recovered, and three weeks later went down with benign tertian malaria, trophozoites and schizonts of *P. vivax* being present in his blood. We mention these three cases because of the view entertained in some quarters (Ingram Johnson 1921, 1923), that in some vague way dengue and malaria are antagonistic diseases. In one instance an infant three days old contracted dengue, the mother also going down with the disease within a few hours of its onset in her infant.

The personnel and establishment at the School and Hospital suffered wholesale from dengue, and durwans, animal attendants, ward boys and sweepers would visit the laboratory to be bled within the first 4 to 6 hours of the onset of fever and symptoms.

Direct Blood Examinations.—The blood of 32 typical cases of dengue was examined by the following technique:—

(*a*) Air dried and Leishman-stained films were searched. No parasite of any type was encountered.

(*b*) Under aseptic precautions a small drop of blood was mixed on a thin glass slide with a larger drop of sterile citrate saline, covered with a thin cover slip, the preparation ringed with vaseline and searched under dark ground examination for not less than 15 to 20 minutes. Examination was with a Leitz 1|12th inch oil immersion lens with funnel stop and No. 6 periplanatic ocular; the No. 15 periplanatic ocular being substituted if anything suspicious was encountered. In most cases two such preparations were searched from each patient, in many from 4 to 6 per patient.

The results were that in 10 out of these 32 cases there were found in very scanty numbers motile forms which appeared to be typical leptospiræ. They varied in hardly any particular other than length. They seemed

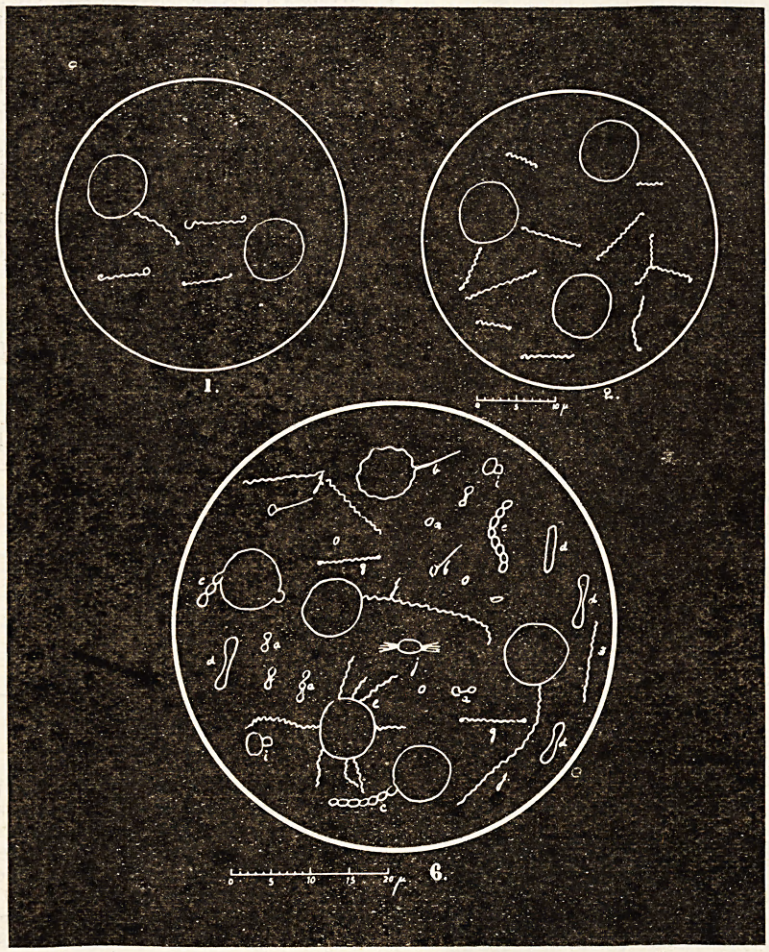


Fig. 1.—Leptospira-like form seen by Col. Megaw in the blood of a dengue case. Sketches of the same form seen at different intervals of time.

Fig. 2.—The leptospira-like artifact described in the text; as seen on dark ground examination of dengue bloods, cultures and the blood of inoculated animals.

Fig. 6.—The puzzles and fallacies of dark ground examination of blood.

(*a*) The hæmoconia particle. (*b*) The straight non-motile streamer. (*c*) The chain streamer. (*d*) The pessary body. (*e*) The multiple short streamers. (*f*) The long, motile, filamentous streamer. (*g*) The leptospira-like artifact. (*i*) Yeasts. (*j*) A short motile bacillus.

to be actively motile with movements of forward and backward progression, often diving down into the fluid and then coming up again as is so characteristic of spirochæte movement. The length varied from 4 to 20 μ, but was usually about 8 to 9 μ. Of 56 specimens roughly measured with the ocular micrometer whilst still motile the average length was 7.8 μ. As a rule

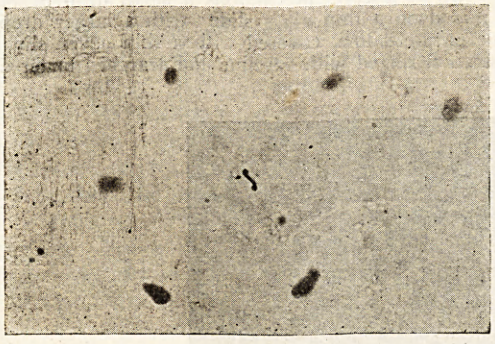


Fig. 3.—Microphotograph of the leptospira artifact from a culture. Short form. Schaudinn's fixative; iron hæmatoxylin stain.

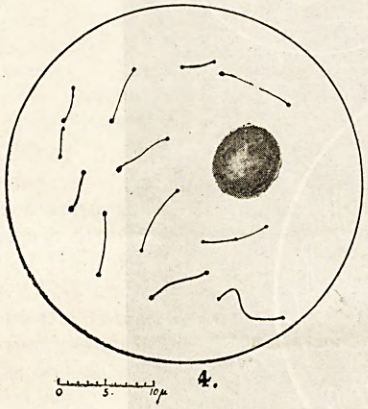


Fig. 4.—Camera lucida drawings of the same from a culture. Same fixative and stain.

Fig. 5.—Microphotograph of the same artifact, long form, as produced in heated non-dengue blood. Same fixative and stain.

these forms were brightly refringent when compared with the pseudo-spirochætes or erythrocyte streamers, but the appearance with regard to this point varied considerably with the lighting. They were fairly flexible, but less flexible than *T. pallidum* or *T. pertenue*. About 10 to 12 very regular and symmetrical turns would be present in a form 8 to 9 μ in length.

The most characteristic feature of these forms, however, was the *invariable* presence, either at one, but almost always at both ends, of what appeared to be a bead or spore. These beads were spherical, larger in diameter than the body of the ? organism, and much more refractile. They appeared as if loosely adherent to the ends of the spirochæte. Figure 2 illustrates the appearances presented by this leptospira-like form when studied under dark ground illumination.

These forms were found in dengue bloods (*a*) in perfectly freshly drawn blood within five minutes of shedding and in the first or second field examined; (*b*) in the complete absence of all such artifacts as erythrocyte streamers, pseudo-spirochætes, etc.; indeed on several occasions where these leptospira forms were found the films were searched for prolonged periods for such artifacts without their being encountered; (*c*) together with such artifacts in blood which had been under examination for some 15 to 20 minutes. Finally they were always present only in very scanty numbers, from 2 to 6 per dark ground preparation. Also the findings were consistent, if one drop of blood from a patient was positive, the same forms would be encountered in the next two or three preparations from the same patient.

With regard to the duration of fever in these cases the findings were as shewn in Table I.

TABLE I.

Direct Blood Examination of Dengue cases.

Duration of fever.	No. of cases examined.	No. positive.	No. negative.
Less than 12 hours ..	11	3	8
13 to 24 hours ..	6	3	3
2nd day	11	3	8
3rd day	3	1	2
7th day	1	0	1
Totals ..	32	10	22

With regard to these cases the positive case at the 3rd day was a patient whose temperature had just dropped to normal. The 7-day case was a patient who developed severe jaundice without fever on the 6th day. 200 c.c. of his urine was centrifuged in a sterile tube by centrifugalisation of repeated 20 c.c. samples in the same tube, and the deposit thoroughly searched under the dark ground. No spirochætes were found. In another case with two days fever the urine was also examined by the same technique but nothing found. In one patient, whose blood has just shewn positive findings and who had an enlarged spleen, spleen puncture was carried out, but no spirochætes were detected and no parasites upon culture. Liver puncture was also carried out in one patient at the 6th day of dengue, with cultures taken, but again with entirely negative results.

We next did our best to try and improve upon these findings. From positive cases, series of blood films were taken, stained by different methods and examined. In four positive cases blood was collected, allowed to clot in the 37°C. incubator and the serum examined at intervals up to 48 hours. No spirochætes were detected. In the case of a patient whose finger blood had just yielded positive findings, blood was taken from the vein into citrated saline and heavily centrifuged and the three layers examined. No spirochætes could be detected in the supernatant fluid or in the leucocyte layer, but they were present in fair numbers in the erythrocyte deposit. This was again repeated on two further positive cases; one shewed similar findings, the second shewed the organism present in all three layers.

To facilitate further reference we may add that the positive findings occurred in serial cases Nos. 5, 8, 9, 11, 13, 14, 24, 25, 26 and 28.

Blood Cultures in Dengue Cases.—Blood cultures were taken simultaneously with the direct blood examinations. At first we used the medium suggested by Kaneko (1921) for *L. hebdomadis*, viz., one c.c. of rabbit blood added to 5 c.c. of Ringer's fluid at 45°C. on the water bath. From three positive cases and one negative case (Nos. 9, 11, 12 and 13) sixteen cultures were put up, incubated either ærobically or anærobically at 22° C. and at 37° C. and repeatedly examined up to 21 days. 14 of them remained sterile; none shewed any leptospira forms in culture.

We turned therefore to the modified Wenyon-Noguchi medium used by Whittingham (1922) for the leptospira found in his cases of sandfly fever, following his technique for pH standardisation and using at first rabbit blood, later human blood, in preparing the culture medium. We did not, however, obtain the rabbit's blood by the method advocated by Wenyon (1921). We have repeatedly tried in Calcutta to prepare media containing rabbit blood by venipuncture of the marginal vein of the rabbit's ear as advocated by Wenyon, and in every instance sepsis has resulted. In fact in the septic atmosphere of a laboratory in Calcutta during the monsoon season with numerous animals being brought into the laboratory daily for examination this method is quite unsuitable. Instead we bleed the rabbit by cardiac puncture, using in the 20 c.c. Roux syringe employed the minimum amount of citrated saline needed to prevent clotting, and then dropping the blood drop by drop from the syringe into the melted medium. We take it that the essential feature of Wenyon's modification is to get a tube with a network of fibrin, and this is obtained by the method which we adopted. Sepsis indeed is a special difficulty in all cultural work in Calcutta during the monsoon period, and we found it necessary to autoclave all media twice and to keep all tubes prior to inoculation in the 37° C. incubator.

TABLE II.

Primary Blood Cultures from Dengue cases.

Medium employed.	Inoculated from case.	Result of examining patient's blood.	Total No. of cultures.	AEROBIC. 22° C.	AEROBIC. Room temp.	AEROBIC. 37° C.	ANAEROBIC. 22° C.	ANAEROBIC. Room temp.	ANAEROBIC. 37° C.
W. R. ..	14	+	6	1+	1+	1×	1-	1+	1-
W. R. ..	15	-	2	..	1+	..	..	1×	..
W. R. ..	16	-	4	..	2+	..	..	2×	..
W. R. ..	18	-	2	1-	1-	..	..	..	..
W. R. ..	23	-	6	1×	1+	1×	1×	1×	1×
W. R. ..	24	+	2	1+	..	..	1-	..	..
W. R. ..	25	+	2	1+	..	..	1+	..	..
W. R. ..	26	+	4	1+	1+	..	1+	1-	..
W. H. ..	27	-	3	1+	..	..	2-	..	..
W. H. ..	28	+	4	1+	1+	..	1+	1+	..
W. R. ..	28	+	4	1-	1+	..	1-	1-	..
W. H. ..	29	-	4	1+	1-	..	1-	1+	..
W. R. ..	29	-	4	1-	1-	..	1+	1-	..
W. H. ..	30	-	4	1+	1+	..	1-	1-	..
Glucose broth.	31	-	2	1-	..	1-	..	..	..
Summary 24 cultures out of	+		53	8+	9+	0+	4+	3+	0+
From cases with blood findings +			22	5+	4+	0+	3+	2+	0+
From cases with blood findings -			31	3+	5+	0+	1+	1+	0+

NOTE.—W. R.-Whittingham's modification of Wenyon-Noguchi medium made with rabbit's blood and standardised to pH 72

W. H.-The same, prepared however with human blood from non-dengue persons in place of rabbit blood.

+ indicates that leptospira-like forms were found, — that none were found and the culture remained sterile, × that the culture went septic.

Twenty-four out of 53 tubes inoculated from 13 typical cases of dengue shewed forms exactly similar to those encountered on dark ground examination of dengue bloods. These commenced to appear in culture about the 5th day, seemed as a rule to be most numerous about the 8th or 9th day, and then seemed to disappear, although one tube remained positive for 28 days. At no time could any culture be termed "rich," the forms seen in these cultures appeared to be considerably more numerous than those seen on direct blood examination, about 5 to 20 per dark ground preparation, but multiplication seemed to be very slow indeed, and it appeared as if we were dealing with a true leptospira, but had not yet struck the best culture medium for it.

We next did our best to improve upon these results, transferring positive tubes from 22° C. or from room temperature to the 37° C. incubator; trying centrifugalisation methods in order to obtain concentration of the virus; using media prepared with ascitic fluid in place of blood and adding sterile rabbit and guinea-pig kidney, etc., but with no results. Sub-cultures on these media were tried, and also 4 batches, each of 6 tubes, of sub-cultures on Whittingham's medium from positive primary cultures. Cultures were also plated out on glucose agar and on blood agar plates. In none of these, however, did we find any leptospira forms.

Hitherto all attempts at obtaining stained preparations of the leptospira form seen had been fruitless. On centrifuging positive cultures and preparing films, however, we at last succeeded in obtaining stained preparations. The methods of fixation and staining used were: (*a*) wet fixation with Schaudinn's fixative, followed by Haidenheim's iron-hæmatoxylin method of staining, which is admittedly one of the best procedures for true cytological study. (*b*) Prolonged staining with very dilute Giemsa's stain after fixation with osmic acid vapour and methyl alcohol. (*c*) Fontana's stain after preliminary fixation with methyl alcohol. (*d*) Leishman's stain. Method (*c*) we discarded early as the resulting film is so full of deposit on fibrils of fibrin, etc., that one cannot be certain of what one is looking at.

Stained preparations by methods (*a*) and (*b*) having been obtained, however, were now carefully searched, and here a surprise awaited us. The forms seen in stained preparations were always exceedingly scanty, far more so than those seen under dark ground examination of the same material, some 3 to 4 per slide only. In all we have seen and studied some 40 of these forms as seen in preparations stained chiefly by method (*a*), whereas we have seen hundreds under dark ground illumination. But whereas under dark ground illumination the forms seen appeared to be *typical* leptospiræ, in the stained preparations they shewed little or *no resemblance to leptospiræ at all.* With iron-hæmatoxylin staining the density of staining varies very much, often the organism is completely decolourised, many stain faintly but with the terminal beads staining deeply, a few stain deeply. Figure 3 is an untouched microphotograph of such a form from a positive primary culture from a dengue blood.

With Giemsa's stain the terminal beads stain deeply blue and the body of the organism deeply pink. With Leishman's stain the terminal beads stain deeply red and the body of the organism a pale violet. In a few instances the organism appeared to be almost straight and resembled a bacillus with metachromatic staining. In almost every specimen seen there is a sinuous curvature, the terminal beads are very prominent, and the stained forms very much recall the old English letter ſ, the body being thin and the terminal beads staining

deeply. The appearance of minute elementary spirals running throughout the body, so characteristic of leptospira spirochætes and so well seen with this pseudo-organism in dark ground preparations, was *not* to be discovered in the stained preparations. Figure 4 of the plate illustrates the forms met with in stained preparations. We now began to wonder whether the organism with which we were dealing was indeed not a spirochæte at all, but a spirosoma or some similar form.

Animals inoculated with the blood of dengue cases.—Animal inoculation was resorted to throughout and every attempt made to transmit the virus to animals and to establish an animal strain for further experimental work. The findings for the 24 inoculated and 2 re-inoculated animals are shewn in Table III. The results are of considerable interest. (The controls will be dealt with later).

What we found in general was that after intravenous inoculation of dengue blood into an animal the first result was an immediate reactionary rise of temperature. Thus rabbit No. 12 shewed a rectal temperature of 103° F. just before inoculation, but an hour after the injection of 4 c.c. of dengue blood intravenously its rectal temperature was 105.4° F. Rabbit No. 13 shewed a temperature of 101° F. just before inoculation; it then received 4 c.c of citrated blood from dengue case No. 11 intravenously plus 4 c.c. of the same blood intra-peritoneally; an hour later its rectal temperature was 104° F. This is a well-known phenomenon and is probably due to the introduction of foreign proteids into the blood stream. The phenomenon is not constant, however, as it often fails to occur.

After this, the temperature drops usually a little, or —sometimes—considerably. Thereafter, however, the animals shewed for from 10 to 30 days an irregularly febrile temperature chart. Considerations of space here prevent our going into details, but the temperature chart of rabbit No. 15 which is shewn as Chart II is typical of the whole series in inoculated rabbits and monkeys (but not of the inoculated guinea-pigs). Despite their apparently febrile state, these animals shewed no other apparent symptoms.

TABLE III.

Animals inoculated with Dengue Bloods or Dengue Cultures.

Animal.	Dose given.	How given.	Donor.	Findings in donor's blood or culture.	No. of days during which animal was subsequently observed.	Blood findings in animal after inoculation.	REMARKS.
M 1	5 c.c.	I.V.	Case 1	−	31	5 times + on 22 examinations.	
M 2	4 c.c.	I.V.	Case 2	−	16	Once + on 12 examinations.	Culture from Case 16.
	3 c.c.	I.P.	Culture	+	10	Never + on 7 examinations.	
M 3	5 c.c.	I.V.	Case 3	−	31	Once + on 16 examinations.	
M 4	5 c.c.	I.V.	Monkey 1	+	41	Twice + on 18 examinations.	Passage.
M 5	5 c.c.	I.V.	Case 8	+	31	3 times + on 21 examinations.	
G P. 1	5 c.c.	I.P.	Case 1	−	14	Never + on 11 examinations.	
G P. 2	5 c.c.	I.P.	Case 2	-	13	Never + on 10 examinations.	
G P. 3	5 c.c.	I.P.	Case 16	−	11	Never + on 7 examinations.	
R 1	2 c.c.	I.V.	Case 2	−	11	Once + on 8 examinations.	
R 2	2 c.c.	I.V.	Case 3	−	11	3 times + on 9 examinations.	Passage.
R 3	5 c.c.	I P.	Case 4	−	30	10 times + on 22 examinations.	
R 4	5 c.c.	I.P.	Case 5	+	6	Twice + on 5 examinations.	Died, 7th day.
R 5	5 c.c.	I.P.	Rabbit 1	+	18	Once + on 14 examinations.	Passage.
	5 c.c.	I.P.	Case 23	−	10	Once + on 8 examinations.	
R E	3 c.c.	I.P.	Culture	+	10	Never + on 7 examinations.	*Vide* Table VI.
R 7	5 c.c.	I.P.					
	2½ c.c.	I.V.	Case 7	−	2	Twice + on 2 examinations.	Passage. Killed 2nd day.
R 8	5 c.c.	I.P.					
	2½ c.c.	I.V.	Rabbit 7	+	25	4 times + on 18 examinations.	
R 9	5 c.c.	I.P.					
	2½ c.c.	I.V.	Rabbit 2	+	29	3 times + on 13 examinations	
R 10	5 c.c.	I.V.	Case 9	+	12	Once + on 8 examinations.	Died 12th day.
R 11	5 c.c.	I.V.	Case 10	−	18	4 times + on 14 examinations.	Died 19th day.
R 12	4 c.c.	I.V.	Case 11	+	11	5 times + on 7 examinations.	Died 11th day.
R 13	4 c.c.	I.V.					
	4 c.c.	I.P.	Case 11	+	18	5 times + on 13 examinations.	
R 14	5 c.c.	I.V.	Case 12	−	23	5 times + on 16 examinations.	
R 15	5 c.c.	I.V.	Case 13	+	23	7 times + on 17 examinations.	
R 16	4½ c.c.	I.V.	Case 15	−	23	9 times + on 15 examinations	
24 animals.					18 (average	75 times + on 320 examinations	

Notes.—M., monkey. G. P., guinea-pig, R. rabbit. I. P., intra-peritoneally. I. V., intravenously. The bloods used were all citrated.

Turning to the findings in these experimental animals:—

(*a*) Three guinea-pigs inoculated intra-peritoneally with the blood of dengue cases Nos. 1, 2 and 16 never shewed any leptospira forms on blood examination. Their temperatures ranged from 102° to 104°F, with a mean on 30 observations at 102.8°F. The normal rectal temperature of the guinea-pig is stated by Archibald (1921) to range between 38.5 to 39.4°C, (101.3 to 102.9°F.) with a mean at 38.7°C. (101.7°F.); and these animals can scarcely be regarded as having been more than slightly febrile. On 28 examinations of their blood no leptospira forms were ever seen, and it would appear as if the guinea-pig was relatively insusceptible to experimental infection with dengue, although relatively susceptible to infection with other

leptospira infections,—a point also noted by Chandler and Rice.

(*b*) Of four adult *M. rhesus* monkeys inoculated intravenously with the blood of dengue cases all subsequently shewed fever or apparent fever. The temperatures ranged from 100.2 to 106°F., with a mean on 76 observations of 102.9°F. Archibald (*loc. cit.*) quotes a range of from 94.8 to 104°F. with a mean at 101.1°F. for the rhesus monkey; and in general these four monkeys appeared to run an irregularly febrile chart for the 10 to 31 days that they were under observation. Chart I is typical of the findings in these monkeys. All of them shewed the same typical leptospira-like forms on blood examination,—10 positive findings on 69 examinations.

In addition to this monkey No. 2, inoculated intraperitoneally with a dengue culture shewing leptospira forms, ran for 10 days a temperature oscillating from 101.6 to 104°F., with a mean at 103.2°F., but shewed no leptospira forms on 7 examinations. Monkey No. 4 was a passage monkey from the venous blood of monkey No. 1 at a moment when the latter's blood shewed leptospira forms. For 41 days it ran an irregularly febrile chart, ranging from 102.6 to 104.6°F., with a mean at 103.5°F.; it also shewed leptospira forms on 2 out of 18 examinations.

(*c*) Thirteen rabbits were inoculated either intravenously or intra-peritoneally or by both routes simultaneously with the blood of dengue cases. All of them subsequently shewed apparently irregularly febrile temperature charts for periods up to a month, and all of them shewed the same leptospira-like forms in their blood. The normal temperature of a rabbit is given by Archibald (*loc. cit.*) as from 37.3 to 39.9°C (99.1 to 103.8°F.), with a mean at 39.2°C. (102.6°F.). Chart II is a typical example of the findings in these rabbits. These 13 rabbits were observed over periods ranging from 2 to 30 days and shewed temperatures ranging from 102.4 to 107°F., with a mean on 139 observations at 103.9°F. Leptospira forms were encountered on 45 out of 144 examinations.

In addition to this rabbit No. 5,—an intravenous passage from rabbit No. 1 at a moment when the latter's blood was positive,—shewed temperatures ranging from 102.4 to 104°F., during the next 18 days, with a mean at 103.4°F., on 14 observations; leptospira forms were only seen once in 14 observations,—on the 7th day after inoculation. Rabbit No. 8, a passage from rabbit No. 7 when the latter's blood was positive, shewed a chart ranging from 102.6 to 104.6°F., with a mean at 103.6°F. on 17 observations during the next 25 days, and leptospira forms on 4 out of 18 examinations. Rabbit No. 9, a passage from rabbit No. 2 at a moment when the latter's blood was positive, shewed temperatures ranging from 102.8 to 107°F., with a mean at 104.2°F., on 13 observations. This rabbit for some reason shewed rectal temperatures of 107°F. and 106°F. on the 27th and 29th days after inoculation, with typical leptospira forms present on both days. Rabbit E, inoculated with a positive 28 days culture, shewed temperatures varying from 103.4 to 106.6°F. during the next 10 days with a mean at 105.2°F., on 7 observations, but no leptospira forms on 7 examinations.

The reader may ask why we did not concentrate our attention only upon such dengue cases as shewed positive findings on blood examination, and inoculate animals only with such selected material. The reasons why we did not do so were two-fold. First we were anxious to work with consecutive, unselected material; as long as the patient shewed typical symptoms of dengue he was used. In the second place the pressure of work with only two investigators and one laboratory assistant was such that whilst one worker was examining the peripheral blood the other did venipuncture and carried out the inoculation of animals and cultures, and we frequently did not know whether the blood was positive or negative until after the inoculations had been carried out. As a matter of fact all the monkeys and rabbits inoculated from dengue cases, whether such cases did or did not shew the leptospira forms in their blood, subsequently shewed what was apparently fever of an irregular type, and all yielded positive leptospira findings. It also appeared as if those inoculated from dengue cases whose blood was positive gave a heavier incidence of positive findings, 32 per cent., than did those inoculated with negative bloods, 29 per cent., as will be seen from Table III.

Four animals in the series died, viz., rabbits Nos. 4, 10, 11 and 13. These were most carefully examined post-mortem and their heart blood and emulsions of the viscera searched for leptospira forms. None were encountered. The cause of death could not be established in any of these four animals, but a mortality of 4 out of 24 animals kept under experimental conditions for periods of from 10 to 40 days probably does not exceed the normal.

Every attempt was next made to secure permanent stained preparations from the blood of the positive animals, using thin and thick films, trying centrifuge methods, etc., but—as with the human bloods—we again failed to get stained films of the leptospira forms encountered. Rabbits Nos. 2 and 7 were killed at times when their blood shewed leptospira forms present and their cardiac blood immediately passaged into fresh rabbits. A thorough search of emulsions of all viscera,—liver, lungs, spleen and kidneys—and of the urine present in the bladder failed to shew any leptospira forms.

Cultures from positive animals.—From such inoculated animals as proved positive we next attempted to recover the organism in culture. The results are shewn in Table IV.

TABLE IV.

Cultures from the blood of Animals Positive in Table III.

Medium used.	Inoculated from animals.	Total No. of cultures.	AEROBIC.			ANAEROBIC.		
			22°C.	Room Temp.	37°C.	22°C.	Room Temp.	37°C.
K. ..	M. 1	4	2—		2—			
K. ...	R. 1	4	1—		1—	1—		1—
K. ..	R. 2	4	1—		1—	1—		1—
W. R. ..	R. 9	5	1+	1+	1+	1+	1+	
W. R. ...	R. 12	3		2+1—				
Glucose broth.	R. 13	1			1—			
W. R. ...	R. 14	2	1×		1×			
Glucose broth.	R. 12	1			1+			
W. R. ...	R. 15	2	1—	1+				
Summary (W. R.)		12	1+	4+	1+	1+	1+	0+

Notes.—K., Kaneko's medium. W. R., Whittingham's modification of Wenyon-Noguchi medium made with rabbit's blood. M., Monkey, R., rabbit.

+ Culture shewed leptospira forms, — culture remained sterile but shewed no leptospira forms, × culture became septic.

As with the human dengue cases, here again we failed with Kaneko's medium, but succeeded with Whittingham's modification of Wenyon-Noguchi medium,—8 out of 12 cultures positive. Sub-cultures were next put up, and at last, in one solitary instance, a sub-culture from a positive culture from rabbit No. 9, leptospira forms were found on the 6th day. The other sub-cultures were negative.

In general, with regard to the animals inoculated with the blood of dengue cases, with positive cultures shewing

leptospira forms, or as passages from inoculated and positive animals, we may conclude:—

(1) That guinea-pigs appeared to be absolutely resistant. They shewed neither definite fever nor leptospira findings.

(2) That rhesus monkeys and rabbits, after a preliminary and transient rise of temperature due to the injection of foreign proteid, tended to shew for from the 10 to 40 days during which they were kept under observation an erratic and generally febrile temperature chart with from time to time leptospira-like forms present in the blood in very scanty numbers.

(3) Cultures from the blood of such animals again yielded the same leptospira-like forms from the 6th to the 20th day, and one successful sub-culture had been obtained.

We next investigated the question of any possible correlation between the leptospira findings and the degree of fever present. The findings are shewn in Table V.

of 55 examinations were positive; at 106 to 107°F. 58 per cent. of 19 examinations were positive. The numbers dealt with are too small for any detailed analysis, but there does appear to be a definite correlation.

Preliminary Survey.—Before passing to the extremely important subject of controls, we may here attempt a preliminary analysis of the results to date. It will be seen that, although not proved, the trend of the experimental evidence was so far more and more in favour of a leptospira-like micro-organism being the true causative parasite of dengue. Such a leptospira form had been seen in the blood of 10 out of 32 typical cases of dengue and seemed to shew a special association with the early and acute febrile phase. It had been obtained in 24 out of 53 cultures from dengue bloods and from 5 out of 13 dengue cases (Table II). Of animals inoculated with the blood of dengue cases all 3 guinea-pigs had failed to take, but all 4 monkeys and all 13 rabbits had shewn an erratic but febrile temperature chart with from time to time scanty lep-

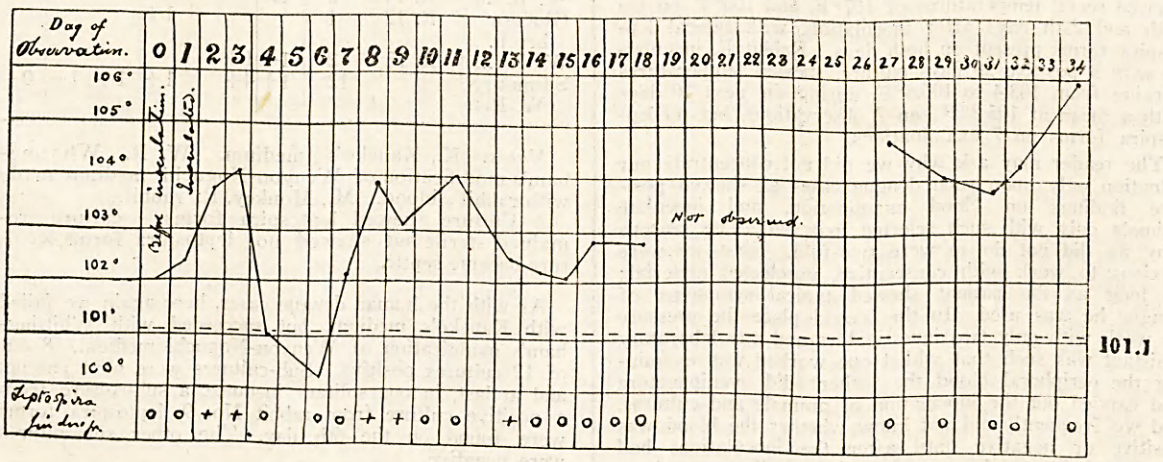
TABLE V.

Inoculated Animals. Correlation between Leptospira Findings and Temperature.

Temperature.	Monkeys.			Rabbits.			Combined.		
	+	−	Total.	+	−	Total.	+	−	Total.
100 to 101°F.	0	4	4	0	1	1	0	5	5
101 to 102°F.	3	5	8	0	0	0	3	5	8
102 to 103°F.	5	38	43	0	12	12	5=9 %	50	55
103 to 104°F.	5	26	31	11	45	56	16=18%	71	87
104 to 105°F.	1	5	6	31	43	74	32=40%	48	80
105 to 106°F.	0	0	0	14	14	28	14=50%	14	28
106 to 107°F.	0	1	1	11	7	18	11=58%	8	19
Totals ..	14	79	93	67	122	189	81	201	282
Percentage positive ..	16%	....	....	35%	....	....	29%	....	....

It will be seen that there is here a distinct correlation between the degree of fever present and the percentage of positive findings. At 102 to 103°F., only 5 per cent. tospira-like forms in their blood (e.g., Charts I and II). Moreover the positive findings appeared to shew a definite correlation to the degree of fever present. From

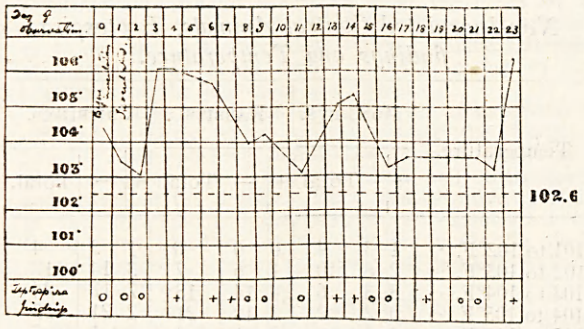
CHART I.



Monkey 1.5 c.c. of citrated blood from dengue Case I. Intravenously.

such positive animals the same leptospira-like form had been obtained in primary blood culture, and once in sub-culture. With the aid of a little *suppressio veri et*

CHART II.



Rabbit 15. 5 c.c. of citrated blood from dengue Case 13. Intravenously.

suggestio falsi, the whole of this memoir could be easily re-written as evidence very strongly in favour of the leptospira theory of dengue.

CONTROLS.

There were certain facts, however, which gave us furiously to think. In the first place the "organism" when stained looked nothing like a spirochæte (figure 4); yet that these stained forms *were* the same as the leptospira-like forms seen under dark ground illumination is proved by the almost invariable presence at their ends of the same bead or spore-like process. Secondly, the erratic character of the temperature charts and the irregular alternation of positive and negative findings in the inoculated animals were puzzling. Thirdly, the variability of the findings in the "positive" cultures, in all of which the leptospira forms seen were scanty, and in some present on 2 or 3 out of 5 examinations, but not discoverable on the other days. From the very beginning our observations were fully controlled, but by degrees the controls became more and more important, until, towards the conclusion of the enquiry, they came to dominate the experimental picture. The control experiments may be grouped as follows:—

(1) *Examination of non-dengue bloods.*—Fresh blood preparations from 10 healthy persons who had not had dengue during 1923 were thoroughly examined for from 15 to 20 minutes each, the technique used being the same as for the dengue bloods. In addition the blood of the senior writer, who had had an attack of what *may* have been dengue in 1922, but who went through the 1923 epidemic without shewing a single symptom of dengue, was repeatedly examined. The serum of 4 non-dengue persons was also examined. In none of these preparations was anything resembling a leptospira form encountered, although pseudo-spirochætes (erythrocyte streamers) were frequently seen.

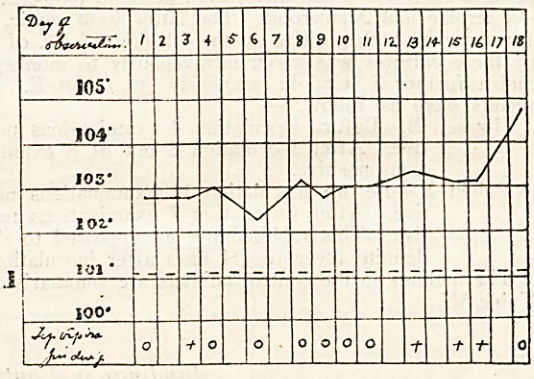
(2) *Cultures from non-dengue bloods.*—Twelve cultures (one each at 22°C., room temperature and 37°C.), in Whittingham medium prepared with rabbit blood were inoculated with the blood of 4 non-dengue persons. Glucose broth cultures from 2 non-dengue persons were taken and incubated at 37°C. A culture from the serum of a non-dengue person was also taken in glucose broth and incubated at 37°C., and 3 sets of cultures from non-dengue sera by a method advocated by Dr. Helen Chambers (1913) in a paper to which further reference will shortly be made. In none of these, although repeatedly examined, was anything like a leptospira encountered.

A culture from the senior writer's blood on Whittingham medium made with rabbit blood and kept at room temperature was found, however, to be positive on the 15th day of culture, and shewed *exactly the same leptospira-like forms* in very scanty numbers on dark ground examination. This was admittedly a most puzzling finding.

(3) *Control non-inoculated animals.*—Eight healthy and non-inoculated guinea-pigs were taken from the run and were examined. Their rectal temperatures varied from 103.2 to 105°F., with a mean at 104.2°F., this being rather high, as two of them, for some inexplicable reason, shewed temperatures of 105°F. A 9th healthy guinea-pig was taken, caged and placed under daily observation. Over a period of 10 days its temperature varied from 100.6 to 104°F., with a mean at 102.5°F. The blood of all 9 guinea-pigs was examined, and that of the 9th again examined on 8 consecutive days. In none of the 16 examinations was anything like a leptospira encountered.

Seven healthy and non-inoculated rhesus monkeys were taken and examined. Their rectal temperatures varied from 101 to 103.6°F., with a mean at 102.7°F. But here a surprise awaited us. On examining their bloods, no less than 3 of them shewed again *exactly the same leptospira forms* as previously encountered in the human dengue cases, with its characteristic beaded ends. Two other fresh monkeys were taken and placed under daily observation. Of these monkey A, observed for 18 days, shewed temperatures ranging from 102.4 to 104.8°F., with a mean at 103.1°F., and with typical leptospira forms present on 4 out of 12 examinations. (Chart III). Monkey B, observed for 7 days, shewed

CHART III.



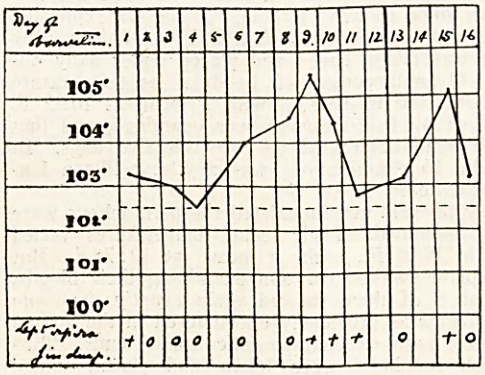
Monkey A. Non-inoculated control.

temperatures ranging from 102 to 103.2°F., with a mean at 102.5°F., but no leptospira forms seen on 4 examinations.

Thirteen healthy and non-inoculated rabbits were taken and examined. Their temperatures varied from 103 to 105°F., with a mean at 104.2°F., and the blood of one of them shewed again *exactly the same leptospira forms as before*. Two non-inoculated rabbits were placed under daily observation. Of these rabbit C, under observation for 16 days, shewed a range of temperature of from 102.5 to 105.6°F., with a mean at 103.8°F., and leptospira forms present on 5 out of 12 examinations (Chart IV). Rabbit D, under observation for 18 days, shewed temperatures varying from 103.2 to 104.8°F. with a mean at 103.9°F., and leptospira forms present on 3 out of 13 examinations. As a further control rabbit E may be included. This was inoculated intravenously with the blood of what was thought at the moment to be a dengue case, but on examining the patient's blood no leptospira forms were seen and microfilariæ were present, and the case was almost certainly one of filarial fever and not of dengue. Rabbit E, kept under observation for 16 days, shewed temperatures ranging from 103.2 to 105.4°F. with a mean at 103.8°F., but neither leptospira nor microfilariæ found on 11 consecutive examinations.

Monkey B and rabbit E, clearly not infected with dengue and with consistently negative blood findings, were next taken and used as critical animals upon which to test positive dengue cultures. The details of these two experiments were as follows:—

CHART IV.



Rabbit C. Non-inoculated control.

(*a*) Serial dengue case No. 16, laboratory sweeper at the School, reported sick with typical symptoms of dengue on the 18th of August. No leptospira found in his blood on examination. A guinea-pig was inoculated intra-peritoneally with his blood with subsequently negative results. Cultures were taken on Wittingham media made with rabbit blood and placed at room temperature, —2 ærobic and 2 anærobic. The latter went septic, the former were both positive on the 9th day. 3 c.c. of one of these cultures was given intravenously to monkey B and a further 3 c.c. intravenously to rabbit E. The results were as follows:—

Monkey B.—Before inoculation 4 examinations negative. After inoculation 5 out of 8 examinations positive.

Rabbit E.—Before inoculation 11 examinations negative. After inoculation 7 examinations negative. This rabbit, however, appeared to shew definite fever for 11 days after inoculation.

The findings in the control animals are summarised in Table VI.

Table VII shews that with regard to the 15 positive and 56 negative findings in the control non-inoculated animals there again appears to be a correlation between the percentage of positive findings and the degree of fever present.

TABLE VII.

Non-Inoculated Control Animals, Leptospira findings and Temperature.

Temperature.	Monkeys.			Rabbits.			Combined.		
	+	−	Total.	+	−	Total.	+	−	Total.
101 to 102°F. ..	1	3	4	0	0	0	1	3	4
102 to 103°F. ..	2	8	10	1	6	7	3	14	17
103 to 104°F. ..	3	3	6	4	14	18	7	17	24
104 to 105°F. ..	0	2	2	2	19	21	2	21	23
105 to 106°F. ..	0	0	0	2	1	3	2	1	3
Totals ..	6	16	22	9	40	49	15	56	71

The figures are too few in number for any definite conclusion on this point, but the temperature chart of rabbit C shews the way in which positive findings seem to go with a high temperature.

(4) *Cultures from non-inoculated control animals.*—The finding of the same leptospira-like form in healthy and non-inoculated animals is in itself surprising enough. We took rabbit C (non-inoculated control), on a day when its blood shewed these forms present and inoculated 4 tubes of Whittingham medium made with rabbit blood, —2 ærobic and 2 anærobic, one each at 22° C. and at room temperature. Both the tubes at 22° C. shewed again typical leptospira forms from the 4th to the 9th days of culture, but both tubes at room temperature remained sterile and negative. Subcultures were attempted from a positive tube but with negative results.

(5) *Summary with regard to Controls.*—The position was now exceedingly puzzling. The blood of 10 non-dengue persons had failed to shew any leptospira-like forms, and 12 cultures from 4 of these persons had remained negative. On the other hand a culture from a person whose blood had been persistently negative and who had entirely escaped dengue in 1923 yielded the characteristic leptospira-like forms on the 15th day of culture. Of 7 non-inoculated monkeys 3 shewed the same form and a non-inoculated monkey, placed under daily observation for 18 days, shewed the same sort of temperature chart as did the inoculated monkeys and leptospira forms present on 4 out of 12 examinations. Of

TABLE VI.

Findings in Control, Non-Inoculated Animals.

Animals observed.	Under observation for	Blood findings.	Extreme range of temperature.	Mean temperature.
8 guinea-pigs ..	Once only ..	None + on 8 examinations ..	103·2 to 105°F. ..	104·2°F.
One guinea-pig ..	8 days ..	Never + on 8 examinations ..	100·6 to 104°F. ..	102·5°F.
7 monkeys ..	Once only ..	3 times + on 7 examinations	101 F. to 103·6°F. ..	102·7°F.
Monkey A ..	18 days ..	4 times + on 12 examinations	102·4 to 104·8°F. ..	103·1°F.
Monkey B ..	7 days ..	Never + on 4 examinations	102 to 103·2°F. ..	102·5°F.
13 rabbits ..	Once only ..	1 + on 13 examinations ..	103 to 105°F. ..	104·2°F
Rabbit C ..	16 days ..	5 times + on 12 examinations	102·5 to 105·6°F. ..	103·8°F.
Rabbit D ..	18 days ..	3 times + on 13 examinations	103·2 to 104·8°F. ..	103·9°F.
*Rabbit E ..	16 days ..	Never + on 11 examinations	103·2 to 105·4°F. ..	103·9°F.
34 animals ..	....	16 times + on 88 examinations	....	....

Note.—* Rabbit E inoculated with the blood of a case of filarial fever, inoculated febrile blood control. *Vide* also Table III.

The temperature chart of rabbit C is given in Chart IV as being typical of the whole series of non-inoculated animals. (This rabbit died, for some reason, on the 17th day of observation, but again a most thorough post mortem examination failed to shew the cause of death and no leptospira forms could be found in emulsions of the viscera.)

13 non-inoculated control rabbits one shewed leptospira forms, and 2 non-inoculated rabbits kept under observation for 16 and 18 days, respectively, both shewed erratic but febrile temperature charts and leptospira forms on 8 out of 25 examinations (rabbits C and D). The "organism" had been obtained in pure culture in Whittingham medium inoculated with the blood of a non-inoculated rabbit, and later the culture had appeared to die out. All attempts to obtain permanent stained preparations had again been negative, owing to the scantiness of the leptospira-like forms.

At this stage we passed through a set of experiments directed towards trying to concentrate the "organisms," taking positive human and animal bloods and positive cultures and laking the blood, and centrifuging and searching the deposit. These had negative results; in fact throughout the enquiry direct examination of the citrated blood or of the cultures under the dark ground yielded a greater percentage of positive findings than did any other method.

FINAL EXPERIMENTS.

We next commenced to examine the tubes of Whittingham medium *before* inoculating them, as a still further control, and were amazed on the 20th of September to discover *exactly the same leptospira-like forms as before* in a tube of culture medium taken straight from the incubator, prepared by the addition of rabbit blood on the 18th of September, but not inoculated with anything at all, "tube 28" of the series.

These findings admitted of only four possible explanations:—

(*a*) That the leptospira-like form was an artifact from the skin or fur of the animals under examination. The fact that it was found in blood obtained by venipuncture (in some instances) of human cases of dengue, that it was entirely absent from guinea-pig bloods, and from all the control non-dengue human and guinea-pig bloods appeared to negative this. Monkey A (non-inoculated control), shewed the typical forms present one morning on blood examination. It was immediately taken, its forearm shaved, cleansed with soap and water, and sterilised in turn with alcohol ether and tincture of iodine. The needle, slides, cover slips and forceps used were all flamed. Three drops of its blood were taken without the addition of any citrated saline and examined direct. In all three the same forms were present in scanty numbers. Whatever the nature of the "organism" seen, it undoubtedly came from the blood and not from extraneous sources.

(*b*) That we were dealing with a "wild" leptospira which occurs in the blood of monkeys and rabbits, but not in that of guinea-pigs. The findings in the human cases negative this. Surely "wild" leptospiræ do not inhabit the human blood in dengue, but not in health.

(*c*) That the control, as well as the experimental animals were themselves going down wholesale with dengue, as well as the human population around them. This appeared to be not impossible. The attendants on these animals, the sweepers, durwans and establishment at the School were going down wholesale with the disease. On the other hand why should guinea-pigs, which are usually held to be especially susceptible to leptospira infections, be so peculiarly immune? On this point we did our best to collect information, and a press communique was issued asking for information as to the possible incidence of dengue amongst the animal as well as amongst the human population. In one instance it was mentioned that a horse and two dogs belonging to a household where there were several cases of dengue had been ill at the same time as the human beings concerned, but in general there was no definite evidence that there was any general epidemic among the animals in Calcutta. The volume of horse and bullock traffic in Calcutta city did not appear to be diminished. Cats were as prevalent and as active as ever at nights, especially in the Park Street area where 6 were caught at the height of the epidemic in a specially devised cat-trap and made over to the Pharmacology Department of the School for experimental purposes; whilst Dr. Bradley, who is an enthusiastic volunteer in the same cause, caught no less than 8 in one night at the Grand Hotel (6 of which were unfortunately rescued by lady residents at the hotel before he could smuggle them down to the School). Further the "positive" monkeys and rabbits did not shew any joint swellings, no rashes were observed, and they did not appear to be suffering from joint pains to judge by the activity of their movements.

(*d*) That the leptospira-like form was an artifact of a peculiar and novel type, only present in the blood during febrile states. The whole trend of the evidence pointed in this direction; the presence of the form in febrile bloods and its absence from non-febrile bloods; its complete absence from guinea-pig bloods, where in our experience artifacts are rare; the erratic and alternating findings in "positive" animals and cultures, both inoculated and non-inoculated; the pseudo-correlation of the form with the degree of fever present; the finding in stained preparations of forms with beaded ends similar to the leptospira-like forms seen under the dark ground, but not true leptospiræ at all; finally the finding of the same form in a non-inoculated tube of culture medium prepared with rabbit blood.

A set of experiments was therefore devised to test the validity of this last hypothesis. It was clearly useless to inoculate any human volunteers or experimental animals with the primary cultures, since in these the original dengue virus might easily have survived merely and not have multiplied. Also it seemed of little use to inoculate even subcultures into men or animals in Calcutta city, since, if dengue resulted and leptospira forms were found in the blood, one would not know whether the infection had resulted from the inoculation or had been acquired through natural channels. Certain experiments were, however, feasible, and they were as follows:—

(1) Tubes of Whittingham medium were made with human blood from persons who were free from dengue in 1923, in place of preparation with rabbit blood. These are the tubes marked "W. H." in Table II, and they were inoculated with dengue bloods. Of 15 such tubes inoculated from 4 dengue cases 9 shewed the same leptospira-like form again. The organism could not therefore be a "wild" leptospira of monkeys and rabbits.

(2) Subcultures were taken from positive tubes into flasks of glucose broth and these were incubated, some ærobically and some anærobically, at 37° C., to test the possibility of the form being a bacillus of aberrant type. No growth resulted.

(3) The junior writer having gone down with a severe attack of dengue during the enquiry was of no value as an experimental animal. The senior writer, however, seemed a suitable animal. His blood had been repeatedly examined with negative results. He had entirely escaped the disease. "Tube 28" (non-inoculated tube prepared with rabbit blood) shewed what was undoubtedly the same organism, whatever its true character. This might be the leptospira of dengue accidentally present and derived from the blood of the rabbit used in preparing the medium, or it might be an artifact. Half a c.c. of the culture was taken hypodermically by the senior writer at a time when dark ground examination shewed it to be positive. Beyond a slight local reaction the next day, the result was completely negative, and not a single symptom of dengue manifested itself. Had the injection been followed by dengue the experiment would have been valueless under the local conditions present, but the completely *negative* result strengthens the case for regarding the form as an artifact.

(4) Three thick and four thin blood films were taken from cases of filarial fever and malaria, stained and searched for prolonged periods in the hope of finding the same forms in stained films from febrile bloods, but none could be found. Only from positive cultures could stained preparations be obtained and even then in extremely scanty numbers. Whatever the leptospira-like form is, it is exceedingly difficult to get stained preparations for study.

(5) On the 11th September the blood of rabbit No. 12 (inoculated intravenously with the blood of dengue case 11, which had shewn leptospira forms on direct examination), shewed fairly numerous leptospira forms. A blood culture was taken from this rabbit into a flask of glucose broth and incubated ærobically at 37° C. On the 10th day the contents of this flask, after centrifuging, shewed the same form present in scanty numbers. A true leptospira, however, should not multiply or even survive under such conditions.

(6) A case of what was clinically typical sub-tertian malaria was obtained from out-patients on the 24th of September. Stained films unfortunately failed to shew parasites, but the case appeared to be typically malaria, and was not at all like dengue. Direct examination of the blood under the dark ground failed to shew leptospira forms. 10 c.c. of blood was taken from the patient, defibrinated, and placed on the water bath at 106° F., (41° C.). Drops were removed from time to time with a sterile capillary pipette and examined under dark ground illumination. At 2 hours erythrocyte streamers commenced to form, but no leptospira forms were seen. At 2½ and 3 hours the blood was full of streamers, both free and still attached to the red blood corpuscles, but numerous and typical leptospira-like forms were present *exactly similar to those previously encountered,* and easily distinguished from the red corpuscle streamers by their more refringent appearance and bright beaded ends.

(7) Five c.c. of blood were taken from a volunteer. The donor has never had dengue and lives in a fifth floor flat where mosquitoes are practically absent. His blood may be regarded as a guaranteed non-dengue blood. The blood was defibrinated and placed on the water bath at 107.6°F. (42°C.), and half hourly observations carried out as before. At 1½ and 2 hours there was marked crenation of the erythrocytes, but no artifacts were seen. At 2½ hours very scanty but typical leptospira forms were seen, but no erythrocyte streamers. The temperature was now raised to 111° F., (44° C.). An hour later the blood shewed a fair number of streamers, whilst the leptospira-like forms were considerably more numerous. Film preparations were taken and stained, some by wet fixation with Schaudinn's fixative and Haidenheim's iron hæmatoxylin stain, others by fixation over osmic acid vapour, methyl alcohol and Giemsa staining. These were studied and again the appearances seen were those of the old English letter ſ. Figure 5 is an untouched micro-photograph from one of these iron hæmatoxylin preparations. With this stain the same characteristic was noticed; that some decolourise entirely, others stain but faintly but with the beads at the ends staining more deeply, whilst a very few stain deeply both as regards the beads and the body of the form.

We conclude that the leptospira-like form found so repeatedly and throughout the enquiry is an artifact of a peculiar type.

Our attention was now drawn by Major Acton to a paper by Dr. Helen Chambers in the *Lancet* of 1913. In this the author describes what she regarded at the time as being a spirochæte present in the blood of the majority of healthy persons. We presume that at this date, ten years later, she will agree with us in regarding that view as an erroneous one. What *are* described in that paper are the different varieties of pseudo-spirochætes met with on dark ground examination of healthy and diseased bloods, and if the paper be read from that point of view it will be seen that it is a most valuable contribution to the scanty literature upon this most difficult subject. The following extracts from Dr. Chamber's paper shew that she undoubtedly encountered the same leptospira-like form in the blood of healthy persons and in cases of goitre in 1913 that we encountered in the dengue epidemic in Calcutta in 1923:—

"The organism is actively motile and is very variable in both length and thickness.....Some are extremely thin, others are almost as thick as typhoid bacilli..... The body is more rigid in the thicker forms.....it is often attached by one end, sometimes by both ends and the body then shows continuous lateral movements. In the thicker forms it shews a fine spiral structure appearing as dark and light closely set transverse lines —waves of motion passing from end to end of the spirochæte can often be detected.....the ends are usually rounded, sometimes they are swollen and look like spores.....round spore-like bodies are sometimes found loosely attached to the ends or laterally on to the spirochæte.

The spirochæte has been stained with Leishman's and Giemsa's stains. Small red chromatin particles are often found at the ends.....the thick forms are easier to stain than are the thin spirochætes, and in stained films the latter are often not detected. The best results with stained films have been obtained from a 48 hour broth culture.....In all the attempts at cultivation the organism has multiplied in the blood itself, or in the first culture taken. It has not, up to the present, been successfully sub-cultured."

MISCELLANEA.

We may here note upon a few miscellaneous experiments not already dealt with. Pemphigus occurred as a clinical complication in not a few of the cases and Dr. Muir very kindly brought us films taken from the contents of the bullæ in such a case in a child. These were stained by different methods and examined. Nothing resembling a spirochæte was seen, the only finding being scanty diplococci, many of them phagocytosed by the polymorphonuclear leucocytes.

Throughout the enquiry we used as a spirochætal control a stock laboratory strain of *S. morsus-muris,* very kindly supplied by Dr. M. J. Permanand, Tutor in Bacteriology, Grant Medical College, Bombay, and kept going by sub-passages in Japanese white mice. In the infected mice we have noted that the spleen is frequently enlarged, often considerably so, and fibrotic, and the best method of passage is to inoculate the mice with *both* the cardiac blood and ground up emulsion of the spleen of the infected mouse. Since 1918 we have come across this organism in five cases of rat bite fever seen in Shillong and Calcutta, whilst Major A. D. Stewart, I.M.S., also kindly presented us in 1922 with a wild rat from Kidderpore docks found infected in nature. On four different occasions we have succeeded in establishing laboratory strains, but in each instance the strain has unfortunately been lost through want of sufficient mice to keep it going. The guinea-pig is an unsuitable substitute; here the infection does take, especially if young guinea-pigs are used, but so scantily that the strain is soon lost in these animals. We have nothing to add to the excellent account of *S. morsus-muris* given by Permanand (1923), except to agree with him that, as is unusual with a spirochæte, the organism is far more easily found in stained films than by dark ground examination. Under the dark ground the longer forms shew a very striking resemblance to the pseudo-organism described in the present paper. We have made repeated efforts to culture *S. morsus-muris* in different media, but so far with negative results.

Transmission.—The pressure of work was such that we were unable to make any organised enquiry into the transmission problem as it affects Calcutta. Members of the staff of the School, however, collected mosquitoes from houses where numerous cases of dengue were occurring and kindly made them over to us. Dr. C. A. Strickland, Professor of Entomology, and Dr. D. N. Roy, Assistant Professor of Entomology to the School, very kindly examined and dissected some of these and allowed us to examine the fresh preparations. One female *Culex* which had not fed recently shewed nothing in either the gut contents, or in the crushed ovaries and salivary glands. Of 9 female *Culex* which had fed recently in dengue houses (whether upon dengue cases or not one cannot say) the gut contents of 4 shewed again the typical leptospira-like form in scanty numbers, varying from 5 to 14μ in length. The crushed ovaries and salivary glands of

3 of them were examined, but nothing found. Dr. Sundar Rao, Filiariasis Research Scholar at the School, also fed a laboratory bred batch of *Aëdes ægypti* on a typical case of dengue on the 29th of September and very kindly permitted us to examine the results of his dissections 21 hours later. In the gut contents of one we found again the same leptospira-like form. We may here remark, however, that if dark ground examination of citrated blood is full of pitfalls and difficulties, that of blood in culture is even more beset with difficulties, whilst when it comes to examination of semi-digested blood from the midgut of a biting insect the task is the most difficult of all. The whole field is full of hæmoconia particles and of pseudo-spirochætes and artifacts.

The junior writer proceeded to Shillong at the end of the enquiry. There had been one reported case of the disease in Shillong: but the patient went down with dengue on the day of his arrival from Calcutta.

Six *Aëdes ægypti* and 20 *Culex quinquefasciatus*, laboratory bred and fed on the 4th October on a typical case of dengue during the first day of fever, were kindly supplied by Dr. Sundar Rao and were taken to Shillong. On the way all the Stegomyia died, and only 9 Culex reached Shillong alive. These were now fed on a volunteer from Tezpur, Assam, an area entirely free from dengue in 1923—the subject never having had dengue. The subject was now placed under careful observation for 14 days. On the 6th day he complained of some headache, and the temperature rose to 99.6° F. for a few hours only. The subject never shewed any symptom really indicative of dengue.

Whilst at Shillong the junior writer again succeeded in producing the same leptospira-like artifact in defibrinated and heated blood from two persons who came from non-dengue areas in Upper Assam, and who had never been exposed to infection with dengue.

SUMMARY.

1. On the examination of blood from cases of dengue; of cultures in Wenyon-Noguchi medium from such bloods; in the blood of rabbits and of rhesus monkeys (but not in that of guinea-pigs) inoculated with dengue bloods; and again in cultures from the blood of these animals, we have repeatedly come across a form which under dark ground examination appears to be a typical leptospira.

2. As seen under dark ground illumination this pseudo-organism is more or less actively motile, it shews up as a rather refringent spirochæte, from 4 to 20 μ in length but with an average length of 8 to 9 μ, and is especially characterised by shewing at one, or usually at both ends, a bright bead—or spore-like process. It is present in only scanty numbers whether in the blood or in culture.

3. In stained preparations we have only been able to identify the same form in preparations from "positive" cultures and never in films from the blood of infected persons or animals. In stained films the organism still shews the typical beads at the ends, but is wholly unlike a spirochæte. It is very much more difficult to find in stained preparations than by dark ground examination.

4. The inoculated animals in general shewed an erratic but febrile temperature chart, but no other symptom of dengue.

5. On examination of blood from control non-dengue persons and control non-inoculated guinea-pigs we completely failed to find the same organism, although it was found in the midgut of fed *Culex* mosquitoes captured in dengue houses, and once in the midgut of an *Aëdes (Stegomyia) ægypti* fed 21 hours previously on a dengue case.

6. In the blood of control non-inoculated monkeys and rabbits, however, we found exactly the same organism. From these animals it was again obtained in culture and in stained preparations from cultures. Either the organism is an artifact, therefore, or else these non-inoculated animals were suffering from dengue acquired through natural channels. They shewed no symptom of dengue other than an irregularly febrile temperature chart.

7. The inoculation of a positive culture into one (presumably susceptible) human volunteer was not followed by any symptom of dengue.

8. We have artificially reproduced exactly the same leptospira-like form in non-dengue bloods, defibrinated and heated for from 2 to 3 hours on the water bath at from 106 to 111° F. (41 to 44° C.). Both under the dark ground and in stained preparations the forms here found correspond exactly with those encountered in the dengue cases, animals, cultures and controls.

9. We conclude that the leptospira-like form encountered so repeatedly is not a true spirochæte at all, but is an artifact, probably especially likely to be encountered when examining febrile bloods and previously described by Dr. Helen Chambers in 1913.

It is humiliating to confess, after two and a half months of strenuous work, that we believe that from first to last we were dealing with an artifact. Yet Major Acton kindly permits us to add that for some weeks he was inclined to believe that we had really discovered a true leptospira in dengue, and an artifact which can *almost* deceive so experienced a worker deserves description, if only as a warning to other workers. Although not the genuine article, it is at least a good *proxime accessit!*

Is dengue then a spirochætal disease? We do not know, but the work of Koizumi, Yamaguchi and Tonomura (1917), that of Chandler and Rice (1923), and the present memoir together constitute fairly strong evidence to the contrary. If the micro-organism of dengue be indeed a spirochæte, one would expect it to be possibly one of *S. morsus-muris* type, possibly vanishing rapidly from the blood at an early stage of the fever to settle in the connective tissues and periarticular structures, where it may possibly remain for some weeks. In such a connection the findings of Couvy (1921, 1922) are very suggestive, but it will be seen that our findings are completely at variance with his.

We believe that the pseudo-spirochæte described in this paper may be not infrequently encountered in examining febrile bloods of both man and animals. In dengue the red blood corpuscles are probably more fragile than normal. In our monkeys, conditions of housing and feeding were not too good, whilst the rabbits in general, for

some inexplicable reason, appeared to be febrile. Under such conditions it is likely that abnormal processes of erythrocyte degeneration should lead to the appearance of pseudo-spirochætes of an unusual pattern. As J. G. Thomson (1923) points out, the supposed "leptospira" of blackwater fever is probably another instance of an artifact being mistaken for a spirochæte.

In conclusion we have to thank Major H. W. Acton, I.M.S., for constant advice, direction, and most helpful criticism. Step by step he studied our findings and the forms met with, and suggested the lines of enquiry to be pursued. Also our thanks are due to Lieut.-Colonel J. W. D. Megaw, I.M.S., for constant help and advice, to Dr. Shivapada Bhattacharjee, M.D. (Calcutta), for taking no end of trouble in securing suitable cases for investigation, and to Mr. Nag, photographer to the School, for the microphotographs.

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*Not studied in the original.

APPENDIX.

I. *On the Rectal Temperature of Experimental Animals in the Tropics.*

We have shewn that we regard the pseudo-organism described above as an artifact. The presence of an artifact cannot possibly cause an animal's temperature to become elevated; but, on the other hand, the injection of a foreign blood may cause a transitory reactionary fever. Hence we took all our temperature records, and from them deleted all readings taken within 24 hours of the inoculation of a foreign blood into the animals. The remaining observations are analysed in Table VIII.

TABLE VIII.

Temperatures of Animals.

Animals observed.	Extreme range of temperatures.	No. of observations.	Mean of observations.
	Monkeys.		
4 inoculated ..	100.2 to 106° F.	72	102.7° F.
No. 2 inoculated with culture.	101.6 to 104° F.	7	103.2° F.
No. 4, passage ..	102.6 to 104.6° F.	19	103.5° F.
7 controls ..	101 to 103.6° F.	7	102.7° F.
Control A. ..	102.4 to 104.8° F.	12	103.1° F.
Control B. ..	102 to 103.2° F.	4	102.5° F.
Ditto, after inoculation with culture.	102 to 104.2° F.	8	103° F.
Total: 15 monkeys	101 to 106° F.	129	102.9° F.
	Rabbits.		
13 inoculated ..	102.4 to 107° F.	126	103.7° F.
No. 5, passage ..	102.4 to 104° F.	14	103.4° F.
No. 8, passage ..	102.6 to 104.6° F.	17	103.6° F.
No. 9, passage ..	102.8 to 107° F.	13	104.2° F.
13 controls ..	103 to 105° F.	13	104.2° F.
Control C. ..	102.5 to 105.6° F.	11	103.8° F.
Control D. ..	103.2 to 104.8° F.	12	103.9° F.
E. inoculated with filarial blood.	103.2 to 105.4° F.	11	103.8° F.
Ditto, after inoculation with culture	103.4 to 106.6° F.	7	105.2° F.
Total: 32 rabbits..	102.4 to 107° F.	224	103.7° F.
	Guinea-Pigs.		
3 inoculated ..	102 to 104° F.	27	102.7° F.
8 controls ..	103.2 to 105° F.	8	104.2° F.
1 control ..	100.6 to 104° F.	7	102.5° F.
Total: 12 guinea-pigs.	100.6 to 105° F.	42	103° F.

It will be seen that our readings are at a definitely higher level than those given by Archibald (*loc. cit.*). The figures run as follows:—

Rhesus monkeys. Extremes, 94·8 to 104°F. Mean 101·1°F. (Archibald).
Extremes, 101 to 106°F. Mean 102·9°F. (our observations).

Rabbits. Extremes, 99·1 to 103·8°F. Mean 102·6°F. (Archibald).
Extremes. 102·4 to 107°F. Mean 103·7°F. (our readings).

Guinea-pigs. Extremes, 101·3 to 102·9°F. Mean 101·7°F. (Archibald).
Extremes, 100·6 to 105°F. Mean 103°F. (our readings).

We have been puzzled throughout the enquiry how to account for this finding. We believe that the thermometers which we used were reliable. In each reading the thermometer was held in place for two minutes as timed by a stop watch. Four thermometers were used at different times but all gave similar readings. We have shewn that we do not believe that epidemic dengue among the animals explains these readings. It is just possible, however, that during August and September, the two months of the year of most intense damp heat in Calcutta, the temperature of such animals *might* be somewhat higher than at other seasons of the year or in colder climates. Their heat regulating mechanism is presumably not quite so well balanced as that of man. On no less than 18 occasions rabbits apparently in the best of health and vigour gave rectal readings of between 106 and 107° F. (Table VI). More information on the subject is badly wanted, and its importance to laboratory workers in the tropics is obvious. We hope to place groups of healthy animals under daily observations over prolonged periods as soon as the newly built animal house at the School is ready, and to analyse the results at a future date. In the meantime we publish Table VIII mainly to draw attention to the matter.

II. *On the Puzzles and Fallacies of Dark Ground Examination of the Blood.*

No one can spend weeks and months together, as we have recently done, on an intensive study of bloods by dark ground examination without gaining a fairly detailed knowledge of this subject. We have not had access to the *Folia Hæmatologica*, but there are two excellent accounts in English of the pseudo-spirochætes and other artifacts encountered in the connection, the best being the classical paper by Dr. Andrew Balfour on blood examination (Balfour 1911), and the other being the paper by Dr. Helen Chambers (1913) already referred to. As the subject is, however, of great importance to laboratory workers we may perhaps be permitted to discuss it here. Figure 6 on p. 13 illustrates the forms most frequently encountered; it is drawn from a collection of sketches and rough notes and drawings made during the course of this enquiry. We believe that such artifacts are much more common in febrile than in afebrile bloods. It is well known that the hæmoconia particles are most conspicuous shortly after a meal when the lipoid content of the blood is raised as a result of the processes of digestion. Also such artifacts tend to increase with the interval of time that elapses between the shedding and the examination of the blood. Further they appear to be much more common near the vaselined edge of the preparation, where osmotic forces are in play, than in the centre.

An excellent way to study such artifacts is to take blood, defibrinate it and then "cook" it for two to three hours on a water bath at a febrile temperature, 106 to 111°F., and withdraw half hourly samples with a sterile capillary pipette for examination under the dark ground. The unheated blood of guinea-pigs appears to be singularly free from such artifacts, whereas they seem to be more numerous in the blood of rabbits and of rhesus monkeys than in that of man. It is important to note that pseudo-spirochætes in very scanty numbers may be encountered within a minute or two of taking the blood and in perfectly fresh material.

We would classify the commoner forms encountered as follows:—

(1) *The ultra-particle.*—The higher the magnification of the ocular used, the more of these that are discovered. They are tiny, ultra-minute particles close upon the limits of visibility, and shew Brownian movement. They are *not* refringent, but dull. If certain of the filtrable viruses be due to organisms of the micro-micro-organism type described by Lieutenant-Colonel H. M. Gordon, R.A.M.C., as the virus of pandemic influenza, then we do not envy the research workers who attempt to observe them under the dark ground, because the ultra-particles will constitute a very great difficulty. In 1922 in conjunction with Major Acton, I.M.S., we spent weeks trying to discover the micro-organism of rabies in the brains of fixed virus rabbits, but were compelled to temporarily abandon the enquiry because the artifacts in an emulsion of brain tissue, whether examined in fresh or in stained preparations, are so numerous that they almost prohibit the discovery of any ultra-minute living organism.

(2) *The hæmoconia particles or blood-dust.* (Figure 6 a).—These are seen in all or almost all preparations. The particle is about half a in diameter, often oval rather than round, and these particles occur either singly, frequently in pairs when they shew a dumb-bell shape, more rarely in chains of three. They shew vigorous Brownian movement. They can always be identified with certainty by one feature; from time to time as they oscillate in the fluid they suddenly catch the light and become for an instant brilliantly refringent, the beam of light resembling the sudden beacon flash from a light-house when seen at sea. These particles may be of lipoid origin.

(3) *The straight, non-motile streamer.* (Figure 6 b).—This is a straight, non-motile, spike-like process originating from the red corpuscles and more frequently from the blood platelets. We have never seen this streamer detached and it could deceive no careful worker. We have recently, however, had submitted to us stained blood films "shewing curious minute flagellate organisms." These were platelets shewing streamers. The straight streamer is *not* refringent, and it is often seen in stained films.

(4) *The chain streamer.* (Figure 6 c.).—These are not infrequent and they are well figured by Balfour. The red corpuscle, during its degeneration, appears to be producing bud after bud, each with its own independent limiting membrane, until a long chain-like form results, swaying in the fluid. We have also seen these chains detached and lying free in the plasma. They are *not* refringent and may simulate yeasts. To what extent true yeasts (cryptococci) are present in the blood of animals from time to time we have not yet determined, but we are convinced that they are not infrequent and that they constitute a special difficulty to workers on *Leishmania* infections in experimental animals.

(5) *The pessary body.* (Figure 6 d).—This probably represents the remains of a distorted or fragmented erythrocyte. Typically it is narrow in the middle and clubbed, ovoid or enlarged at both ends. It shews a well defined limiting membrane and a dull body. The shape may vary from a big bacillus-like form to a dumb-bell type, but it has one diagnostic feature. From time to time as it oscillates in the fluid it suddenly catches the light and becomes for a moment brilliantly refringent, as does the hæmoconia particle. There is the same beacon-like flash about it.

(6) *The multiple streamers.* (Figure 6 e).—Very frequently a degenerating red corpuscle is seen to be giving off simultaneously from 6 to 10 small, fine streamers. Pores appear to form in the erythrocyte membrane and from them the cytoplasm of the cell protrudes in the form of thin, pseudo-motile streamers, usually short and never, we believe, when multiple much

longer than the diameter of the red cell. These forms are *not* refringent and we have never encountered them free in the plasma. We believe that what next happens is that usually one of them grows into the long motile streamer (No. 7) at the expense of the rest.

(7) *The long, filamentous, motile streamer.* (Figure 6 f).—This is peculiar and most characteristic. It is especially met with in blood drawn some time previously, although we have also seen it in perfectly fresh blood. A pore seems to form in the erythrocyte membrane and through it the cytoplasm streams out in the form of a long, flexible, filamentous form closely simulating a very long spirochæte. There is no knob or bead at the end, which is rounded and not pointed. This form may attain enormous lengths, beginning as a small protrusion 4 to 5μ in length it may grow to 25 or even 40μ, and not infrequently appears to stretch from one red cell towards another some distance away. As a rule this streamer is single, but on a very few occasions we have seen two produced simultaneously by the same erythrocyte.

Finally this form becomes detached from the cell. It now appears as if actively motile, twisting and turning about, not infrequently knotted upon itself, and often (probably from the presence of adherent hæmoconia particles) appearing as if shedding granules. It is somewhat refringent, but not markedly so. In a vaselined preparation it will be found that these forms are especially encountered near the edge of the preparation where presumably surface tension effects are maximal. The long thin motile streamer is the commonest form of pseudo-spirochæte met with in blood preparations.

(8) *The leptospira artifact.* (Figure 6 g).—This has been so fully described in the above memoir that we here need only mention it. It may prove of considerable difficulty to workers on leptospira infections. Its motility varies from sluggish to actively motile, its length from 5 to 20μ, but usually 8 to 9μ, although in some preparations short forms, 4 to 5μ are seen. In appearance it simulates a typical leptospira with one important difference, the ends are not looped but beaded and unmistakably so. The body is thicker, more rigid and more refringent than that of No. (7), but the terminal beads are always brightly refringent. From time to time it catches the light and suddenly becomes brightly refringent. Not infrequently it becomes attached by one or both bead-like ends to the slide or cover slip, whilst it also shews the diving movements so characteristic of a spirochæte and often simulates a dead spirochæte. We have never seen it attached to a red corpuscle but it has a special habit of diving under and disappearing from view beneath a red cell. The School has recently purchased a set of cinema films from Messrs. Pathe Frères and one of them is a particularly fine film of a culture of *L. icterohæmorrhagiæ* as seen under the dark ground. We have twice studied this film upon the screen, and so closely does this dengue artifact simulate a leptospira that we could almost identify the film as one from a dengue culture, except for the immense number of parasites present in the field.

(9) *Bacteria* of extraneous origin may be encountered, especially in preparations from furred animals. They are usually unmistakable. One which we have repeatedly seen in blood preparations from rabbits and monkeys is a short, squat little bacillus with two terminal tufts of fine, scarcely visible flagella. It remains motionless for a short time, then suddenly and very rapidly jumps to another spot, sometimes changing direction two or three times in transit, and then stops and becomes motionless again.

If our memoir does nothing else, we hope that it will at least serve as a warning as to the extreme necessity for full controls in all experimental work upon spirochætal infections. The Calcutta epidemic has now ceased (20th October, 1923), and for the present no further work upon the problem is possible at the School.

THE DETERIORATION OF INSULIN IN INDIA.

By J. TAYLOR, D.S.O., M.D., D.P.H.,
MAJOR, I.M.S.,
Offg. Director, Pasteur Institute of Burma, Rangoon.
and
A. R. J. DOUGLAS, M.D., F.R.C.S., D.P.H.,
Chief Medical Officer, Burma Railways, Rangoon.

WE desire to issue a warning as to the use of insulin imported into India under conditions which may permit of deterioration in potency.

Our experience is based on a trial of insulin of American manufacture prepared by one of the first firms to receive a license to make it and of the original potency of which there can be little doubt. The insulin made by this firm has been extensively used in the United States, and reports in the medical journals show that it has been very satisfactory.

Our supply was purchased from a local firm in Rangoon who had obtained it, not direct from the manufacturers, but through a reliable and well-known firm in London. Transit between London and Rangoon was not made in cold storage and the insulin was not kept in cold storage during the three weeks it remained in Rangoon before it was purchased. We have no knowledge of the date of manufacture nor of the conditions of handling before it was despatched to Burma.

The first trial was made on a severe case of diabetes, a European, aged 36, whose condition was first diagnosed in 1920. Prior to that year he had weighed 12¼ stone and by 1921 his weight had come down to 8¼ stone. In the following year he lost another 7 lbs. and had to reduce his diet to 1,180 calories, consisting of carbohydrate 10 grms., proteid 60 grms., fat 100 grms. to keep his urine sugar-free. He became progressively worse, and insulin treatment was commenced on 11th September, 1923. For a few days before the first treatment his diet was raised to 2,000 calories.

The results of his first insulin injection were as follows:—

9 a.m.—Urine Sugar 5.7 per cent.
9-15 a.m.—Blood Sugar 0.51 per cent.
9-20 a.m.—10 units insulin.
9-45 a.m.—Meal of carbohydrate 25 grms., proteid 16 grms., fat 70 grms. (Total calories 794).
10-15 a.m.—Blood Sugar 0.49 per cent.
11-15 a.m.—Blood Sugar 0.39 per cent.
12-15 p.m.—Blood Sugar 0.37 per cent.
Ten units were given in the same evening.
On subsequent days the doses were:—
12th Sept.—10 units and 15 units.
13th Sept.—15 units and 15 units.
14th Sept.—15 units and 20 units. (Diet reduced to 1,700 calories).

Another series of tests were made on 15th September:—

9 a.m.—Urine Sugar 7.7 per cent.
9 a.m.—Blood Sugar 0.48 per cent.
9-30 a.m.—25 units insulin.