Bacteriology. — "*Researches relating to the Etiology of Febris Exanthematicus.* By Dr. C. J. C. VAN HOOGENHUYZE. (Communicated by Prof. C. EYKMAN).

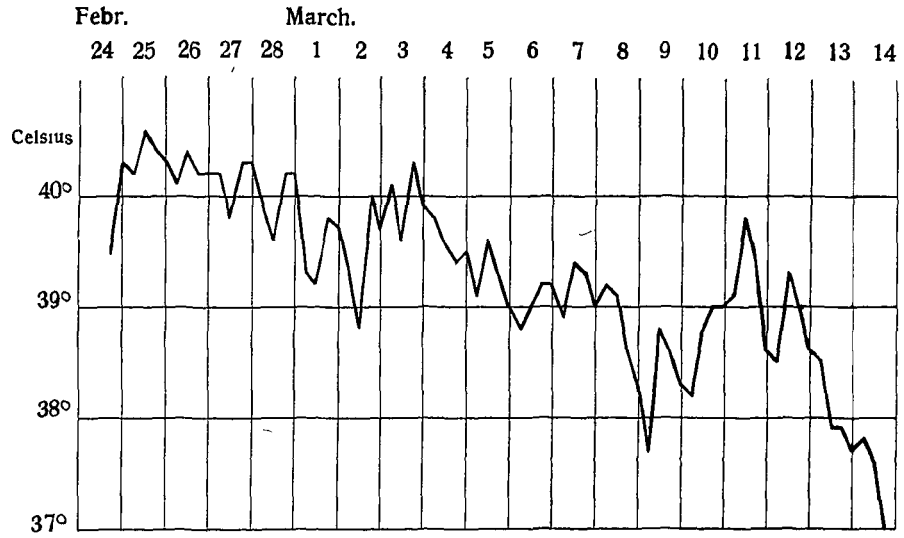
(Communicated in the meeting of June 30, 1917).

Many researchers have been engaged in studying the causation of Febris exanthematicus, and several organisms have already been looked upon as the causative agent of this disease, such as protozoa, spirilla, diplococci and diplobacilli. Considering, that even in such cases in which the researcher succeeded in growing pure cultures from the blood or from an organ, the several findings did not agree, or did not seem to agree in every respect, so that we do not know what to think of them, all these inquiries are looked at askance and it is generally acknowledged that the real etiological factor has not yet been found. Every one pursuing this line of research should, therefore, record his results for the purpose of comparison with those thus far obtained. Fortunately cases of spotted typhus are few and far between in Holland. When, therefore, some cases occurred at Amsterdam a few months ago, I seized the opportunity to study them, and determined to publish my observations for the reasons stated above, although I for one feel convinced that one investigation is not of great value. The patients were treated in the Wilhelmina-Hospital. Through the kindness of Dr. KUIPER, the Director, and of Dr. v. ZADELHOFF, the physician of the ward, I was in a position to examine the blood of one of the patients who was still very ill.

This patient F. W. R., 59 years of age, was admitted to the Hospital on Feb. 24. On the 20th he had had chills. On the 21st he was laid up with head-ache, gripes, and back-ache.

Status praesens: Patient does not look ill, does not complain; throat: somewhat red; tongue: coated and tremulous when put out; full pulse, not dicrotic; Lungs, heart: no anomalies; Bowels: present nothing particular. Patellar reflexes: high; plantar reflexes: normal. Many roseolae on chest, belly, back, arms and legs. WIDAL negative. In the urine much albumin, much urobilin. Sediment: some red, and white blood corpuscles, kidney-epithelium, some cylinders. Blood: number of leucocytes 7000. Temperature: See list I.

Temperature I. Pat. F. R.



On March 2 I drew by means of a syringe ± 20 c.c. of blood from a brachial vein (of course with the necessary aseptic precautions). Immediately I transferred $^1/_2$ to 1 c.c. of the blood to tubes filled with 10 c.c. respectively of tapwater, 0.25 % NaCl, 0.5% NaCl, 0.75 % NaCl., peptonewater, ascites-glycerin-bouillon, (1 c.c. ascites fluid, 9 c.c. of bouillon, 0.4 c.c. of glycerin) and 2 % ammonium oxalate, two tubes of each sort. The fluids were heated to body-temperature and in going from the laboratory to the hospital and vice versa I took preventive measures to insure security from cooling down. I made use of water and several amounts of common salt, because, if NICOLLE, CONOR and CONSEIL are right in asserting that the virus is contained chiefly in the leucocytes, I could thereby liberate the organisms by destroying the leucocytes. According to GOLDBERGER and ANDERSON, however, who made similar experiments to those of the researchers just mentioned, and who pointed to their faulty conclusions, it may be more readily assumed that the virus occurs isolated in the plasma. However this may be, I thought fit to take note of both investigations. I grew at 37° C. organisms both in aerobic and anaerobic cultures. To obtain the latter I put some pyrogallol and potassium-hydrate upon the cotton plug and shut up the tubes with rubber stoppers. Of course I also made the necessary control-experiments.

After intervals of 24 hours preparations were made of all aerobic cultures; the anaerobic cultures were examined only macroscopically, to see if there was any growth. After 3 × 24 hours preparations

were made also of the anaerobic cultures. The aerobic cultures yielded the following results:

Blood in water: a few diplobacilli. GRAM positive.

Blood in 0.25 % NaCl: a few diplobacilli GRAM positive.

Blood in 0.5 % NaCl: a few diplobacilli. GRAM positive.

Blood in 0.75 % NaCl: no micro-organisms.

Blood in peptone-water: no micro-organisms.

Blood in ascitesglycerin-bouillon: a few diplobacilli. GRAM positive.

Blood in ammonium-oxalate: a few diplobacilli. GRAM positive.

From the tubes, in which diplobacilli had been found cultures were made upon sloped agar. 24 hours later everything was examined again. The agar-tubes had all remained sterile. In the other tubes the following was observed:

Blood in water: a few diplobacilli, to all appearance not many more than the first time. GRAM positive.

Blood in 0.25 % NaCl: many diplobacilli. GRAM positive.

Blood in 0.5 % NaCl: many diplobacilli. GRAM positive.

Blood in 0.75 % NaCl: no micro-organisms.

Blood in peptone-water: no micro-organisms.

Blood in ascitesglycerin-bouillon: rather many diplobacilli. GRAM might be called positive as well as negative.

Blood in 2 % ammonium-oxalate: some diplobacilli, not many more than the first time. GRAM positive.

From the tubes in which diplobacilli were found again cultures were made upon sloped agar.

After 24 hours it appeared that besides the remains of red blood-corpuscles, also diplobacilli, very much like those found previously, were present in the liquid expressed from the agar of the agar-tubes, inoculated with the blood + 0.25 NaCl and the blood + 0,5 NaCl, likewise in the liquid of the agar-tubes that had been inoculated the day before. All the other agar-tubes had remained sterile. The preparations of the liquid-tubes were similar to those of the second day.

The anaerobic tubes appeared yet to be sterile.

The question now arose if what had been found in the fluid squeezed from the agar, meant growth or whether it had been evolved by inoculation. I collected the fluid from the several tubes and inoculated it into some sloped-agar tubes, made a stabculture and added broth to the rest to the ratio of 1 expression of water to 10 of broth. After 24 hours, preparations were made of the broth and many diplobacilli were found.

When watching the agar-tubes, especially those that had been

inoculated with 0.25 % and 0.5 % NaCl + blood, the surface did not any more appear to me quite clear and transparent, though I did not see any colonies either. Next day little more was noticeable. However one preparation made of it revealed diplobacilli. I now took a piece of this somewhat turbid-looking agar and ground it in broth.

In the depths of the stabculture something similar was to be seen; preparations of it contained the same diplobacilli. Of this agar I also put some pieces in broth.

Preparations made from these broth-tubes showed numerous diplobacilli. I made a subculture of some of this broth upon sloped agar. Only after 24 hours did I see a few colonies appear. Likewise a few colonies were visible upon the agar inoculated with expression-water, very much like the preceding. Repeated subcultures upon agar resulted in more colonies; though the growth was rather poor, it gradually seemed to progress.

The colonies produced looked white with incident light, were clumped and could be easily shifted bodily with a needle all over the agar-surface. It seemed as if the colonies floated upon the agar. With transmitted light they looked bluish and translucent.

The bacilli are not all exactly alike as to shape. We found short rods and longer ones. The short ones generally occur in pairs joined end to end, so that they might be called diplobacilli. Most often the centre of the longer ones is of a lighter colour, which renders the bacillus dumbbell- or biscuit-shaped. Maybe we have to do here also with two bacilli joined end to end, or all of them are single bacilli depressed in the middle? However this may be, it is evident that the two parts are linked together. In some cases we might even term them diplococci, the cocci being slightly elongated (coccobacilli). See Fig. 1 and 2 of the plate.

The bacilli (I will stick to that term) have no spontaneous motility. They readily stain with the ordinary dyes, most distinctly with carbol-gentian-violet, which, therefore, I made ample use of, also in my investigations of the bacilli in the digestive and intestinal tract of lice. With LÖFFLER's methyleneblue we can see at the poles more darkly stained granules, occasionally distributed all over the body. They are Gram-positive and non-acidproof. In ascites-glycerin-bouillon, and in ordinary broth cultures, especially the older ones, other bacilli are found among the Gram-positive bacilli, that look exactly like them, but are decolorized.

The bacilli are clumped, which renders suspension difficult.

The best temperature for the growth seems to be 37°. At room-

temperature they do not grow at all. At first a culture was made every day; after some experiments the interval could be lengthened, at most to about 3 weeks, this being the longest interval after which growth seems to be possible.

Upon gelatin at 22° the growth is exceedingly tardy. No liquefaction of gelatin takes place.

Upon LÖFFLER's serum: rather poor growth; especially the above-mentioned granules are made out here.

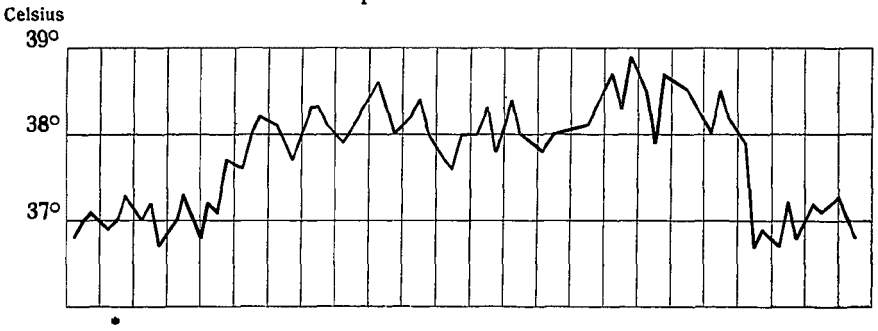
Upon Endo the growth is also poor. The colonies are white and change to light red after a few days.

In bouillon a sediment is formed. The broth does not get evenly turbid, only in the lower layers.

Indol is not produced. Milk does not coagulate. Saccharose-, lactose-, mannite-, glycose-, maltose-, raffinose-bouillon: no acid-formation, no fermentation.

In caviae, inoculated with a suspension of this broth, a rise of temperature was observed after 4 or 5 days, that persisted for a shorter or a longer space of time as is shown in List II.

Temperature II. Cavia.



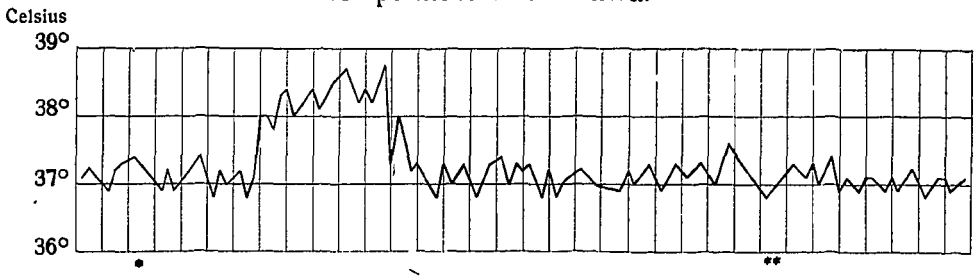
Subcutaneous injection of a suspension of the bacilli.

They then sat quietly in a corner of the hutch and appeared to be ill. To a second injection a short time after the temperature had become normal, there was no response and the temperature remained constant. To all appearance they had become immune as is shown in List III.

In order to make sure that this was not brought about by a less pathogenic condition of the cultures, also healthy caviae were inoculated at the same time. With them a reaction really took place.

We managed to cultivate the same bacilli with the same properties from the blood drawn from the heart of one of the caviae by means of a puncture and that on a day on which the temperature

Temperature III. Cavia.



* Subcutaneous injection of a suspension of the bacilli.
** Inoculated for the second time.

was very high. This experiment was conducted in the same way as described above.

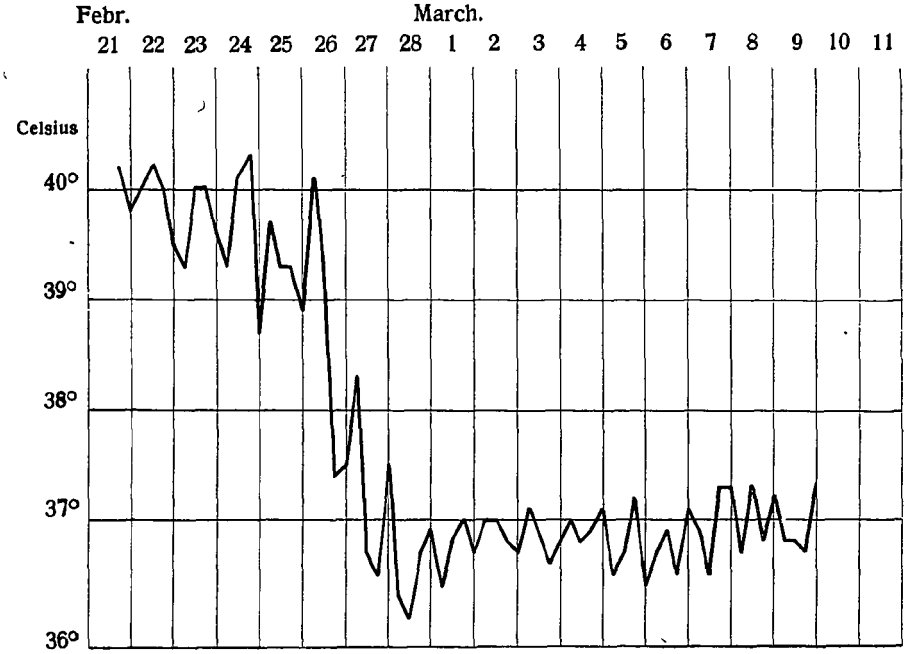
Lastly I have also been able to study the agglutination. On the 9th of March, i.e. 7 days after the blood had been drawn for the culture, I again drew some blood from the patient. The serum agglutinated the bacilli in a 1 : 100 dilution; the serum of two sound subjects and a sick one (with a high temperature) only in a 1 : 25 dilution. In a second patient, who had also suffered from typhus exanthematicus I performed an intravenous puncture 11 days after the crisis (See List IV). I did not succeed in cultivating bacilli from the blood. The serum agglutinated the bacilli described above in a 1 : 100 dilution. The history of the case is briefly as follows:

17 Febr. pat. (M. J. F. V. 21 years) was down with a bad head-ache, gripes, fever, pains in the arms and legs, chills. For about 3 weeks previously he had been languid. *Status Praesens*: pulse: quite full, not dicrotic; Coniunctiva slightly red. Diffuse bronchitis in both lungs; patellar reflexes very high, spleen did not seem to be enlarged, as appeared from percussion, not palpable. Scattered roseolae on chest, back, arms, legs, belly, also some in the face. Hemorrhage from the mucous membrane of the mouth; here and there spontaneous bleeding. The urine presents nothing peculiar. Number of leucocytes 8000. WIDAL negative. Diagnosis: spotted typhus.

It appeared, then, that the serum of both patients, one of whom was recuperating, agglutinated the bacilli in a 1 : 100 dilution; the serum of a patient suffering from another disease with high fever in a 1 : 25 dilution; the serum of sound subjects also 1 : 25.

In connection with the communications of DA ROCHA LIMA and TÖPFER I have examined some clothes-lice, taken from the body-linen of the first patient (R). I prepared as well as possible the digestive and intestinal tract from the body and ground it on an

Temperature IV. Pat. M. V.



object-glass. I was fortunate enough to demonstrate organisms, exactly similar to the bacilli described above and to those described by DA ROCHA LIMA and TÖPFER. On the photographs 3, 4 and 5 some bacilli are distinctly visible. I could not demonstrate these organisms in clothes-lice or head-lice taken from sound persons nor in house-bugs.

The only difference was the staining, after GRAM, as these organisms were easily decolorised (as DA ROCHA LIMA also tells us) whereas the cultivated bacilli were not. For want of material I was not enabled to grow cultures from the organisms found in lice.

Finally I examined several coverslips of the patient's blood, stained after GIEMSA, to detect bacilli and the bodies that various researchers discovered in the white bloodcorpuscles; here and there I saw in some leucocytes in the protoplasm beside the nucleus now punctiform bodies, now again rods with rounded ends, sometimes indented in the centre. This is exemplified in the figures 6, 7, 8, 9. It is especially the latter that remind one forcibly of the organisms described above. Staining with methyleneblue made everything clearer still.

Whether we really have to do here with bacilli which, on account of their being disposed differently in the microscopic field, present different shapes, I will not venture to decide. On slightly turning

the screw of the microscope it seems as if the point is shifted a little, so that it may be taken for a rodlet slightly inclined from the vertical. However this may be, it is at any rate remarkable to observe the resemblance in the shape of the bacilli found in the first fluids, the bacilli cultivated, the bacilli in the intestinal tract of the clothes-lice and the bodies (such I think they may be called) in the white blood corpuscles of the patiënt. Besides these shapes in the leucocytes I also, though rarely, detected a diplobacillus in the coverslips, after a prolonged research.

It must be observed that not in all the fluid tubes bacilli were found, although they all contained blood from the same syringe (with a control for an aseptic operation). They were found only in those tubes in which the fluid was hypotonic relating to the blood; it should also be observed that after some subcultures, the cultivation was considerably easier and the intervals between two successive cultures could be lengthened; again, that the bacilli then seemed to be less closely packed together and less pathogenic.

It is worth while to compare this finding with those of other researchers with numerous material.

In my judgment there is a great resemblance between the organisms found by DA ROCHA LIMA and TÖPFER in the body of lice, without their being able to cultivate them, and those demonstrated by me. Owing to lack of material I was not in a position to perform sections in order to observe the different epithelial cells, as described by DA ROCHA LIMA and was limited to coverslips only. This may account for my organisms lying free in the field of view. Entire epithelial cells I did not see, only fragments of them.

I am personaly inclined to think after consulting the literature, accessible to me, that the bacilli detected by us when compared with cultures grown by various experimenters resemble most those of RABINOWITSCH. For the sake of brevity I shall not pass in review all the researches and only refer to the extensive publications of RABINOWITSCH, who comes to this final conclusion, that in reality all investigators have found one and the same organism, viz. short shapes growing in pairs. The slight deviations recorded. are owing to the different methods of staining. The properties also agree rather well. This is also the case with the bacilli cultivated by me. Only the description of the agar-colonies do not apply to my cultures. According to him they first resemble colonies of streptococci (dew drop) and afterwards assume a pale yellow colour. My colonies are compact, white and remain so. With incident light they remind one of colonies of staphylococci. For the rest they are not at all like them,

as set forth in my description. The properties and animal experiments correspond to those of RABINOWITSCH and others. RABINOWITSCH believes that the reason why so many investigators could not produce a culture, is that the bacilli occur in the blood principally in a certain period, viz. at the conclusion of the fever-period, immediately before the crisis, so that, as he puts it, it is not the day of the beginning of the disease, but the day of the crisis that is of prime importance.

I happened to collect blood from the patient on the very day when his disease had reached its acme and his life was feared for. The following day, however, he suddenly took a turn for the better, but in consequence of several complications occurring with the disease, the temperature was not indicative of the time of the critical period.

Those who have grown these bacilli agree that it is exceedingly difficult to produce cultures. Frequently the rods were demonstrated in the microscopic preparations (e. g. in broth or expression-water), but they could not be developed any farther. RABINOWITSCH holds that the blood-serum of the patients inhibits the growth also when diluted. Very weak dilutions would, therefore, further the growth. Even the fluid expressed from the agar he considers to be a dilution. Without touching the agar surface he first puts the blood in the expression-water and only then, after shaking it well he pours it out over the agar. My success in producing cultures may be owing partly to similar dilutions in my procedure, and partly to the use of hypotonic fluids to put the blood in, and to the lucky circumstance of drawing the blood at the proper moment.

PLOTZ and later on POPOFF are the only investigators who detected an organism considered by them as the etiological factor of typhus exanthematicus, differing essentially from all other organisms detected, this bacillus typhi-exanthematici being obligate anaerobe.

Several investigators succeeded in cultivating organisms not only from the blood, but also from the urine and the feces of sufferers from typhus exanthematicus. They were agglutinated by the blood of the patients. HORIUCHI e.g. produced from the feces and the urine a paratyphus-like bacillus; WILSON obtained from urine and feces bacilli differing from the Bacillus coli only in that they did not convert lactose; PREDTJETSCHENSKY managed to grow from urine, sputum, and bronchial mucus the same diplobacilli as from the blood; KLODNITZKY grew from house-bugs, obtained in a typhus ward, a culture of small motile very virulent bacilli, called by him bacillus violentus. However he refrains from considering them as the agent exciting typhus exanthematicus. PETRUSCHKY collected from sputum rodlets which he took to be the cause of the disease; WEIL

and FELIX grew proteus-like bacilli from urine; DIENES managed to grow from two clothes-lice taken from a patient proteus-like bacilli, resembling those of WEIL-FELIX.

I will not attempt to criticize, as I am fully aware that one case cannot yield conclusive evidence. Still, it favours the supposition that in this patient the bacillus detected was indeed the cause of the disease, which had been clinically diagnosed as "typhus exanthematicus". Also in my investigations questions are still left open, owing to lack of material.

We now summarize our results:

From the blood cultures were grown that may be called diplobacilli on account of the form and the location. The colonies upon agar look white with incident light and faint-bluish transparent with transmitted light. They are detached from the agar surface and can easily be shifted bodily with a needle.

The bacilli possess no motility, take up all dyes and their appearance varies with the dye selected, (finer, coarser, granular, dumbbell- or biscuitshaped). They are Gram-positive (in older cultures occasionally Gram-negative); they are non-acidproof; they form no spores.

No growth at room-temperature. Tardy growth at 22°. They grow best at 37°.

Gelatin is not liquefied; upon LÖFFLER's serum there is only a poor growth; milk does not coagulate; the bacilli do not form acid nor indol. No fermentation in saccharose-, lactose-, mannite-, glycose-, and raffinose-bouillon.

After repeated inoculations the properties change.

They are pathogenic for caviae (i e. they cause a rise of temperature) [could again be cultivated from the blood of these caviae] and render them immune. Through the serum of the patient himself and that of another who was convalescent they were clumped in a 1 : 100 dilution; through the serum of a sufferer from another disease and two sound persons only in a 1 : 25 dilution.

In the digestive and intestinal tract of clothes-lice, taken from the patient, organisms were found resembling the above-mentioned bacilli and the Rickettsia Prowazeki of DA ROCHA LIMA. With them no culture- nor animal-experiments could be made for want of material. The Gram-staining was negative here.

In the coverslips of the blood of the patient very rarely a single diplobacillus was found. True, some forms were detected outside the nucleus in some leucocytes that reminded us forcibly of the above-mentioned organisms (also those in lice).

The Amsterdam Public Health Service Bacteriological Laboratory.

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Other works on this subject were not accessible to me.

Fig. 1.

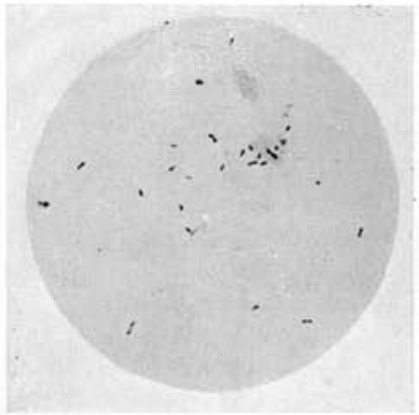

Fig. 2.

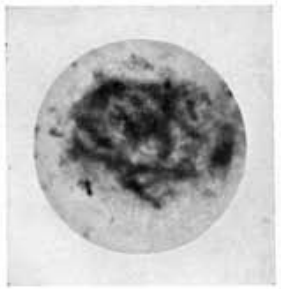

Fig. 3.

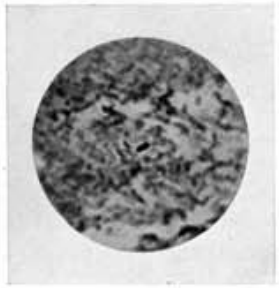

Fig. 4.

EXPLANATION OF THE PLATES.

Fig. 1. First obtained culture from blood of the patient, on agar. (Augm. 1560).
Fig. 2. Culture after inoculation on agar. (Augm. 844).
Fig 3 Preparation of the digestive and intestinal tract from a clothes-louse of the patient, ground on an object-glass. (Augm. 1560)
Fig. 4. The same of a second clothes-louse of the patient. (Augm. 1560).
Fig. 5. The same of a third clothes-louse of the patient. (Augm. 1560).
Fig. 6. (Augm. 1540).
Fig. 7. (Augm. 1560).
Fig. 8. (Augm. 1500).
Fig. 9. (Augm. 750).
} Leucocytes of the blood of the patient with several forms of corpuscles outside the nucleus.

The photos have been made by Dr. L. TH. REICHER, whom I render my sincere thanks.

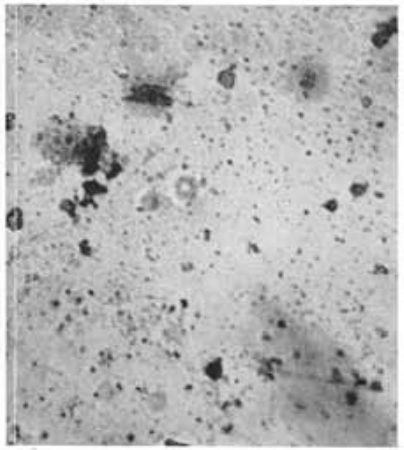

Fig. 5.

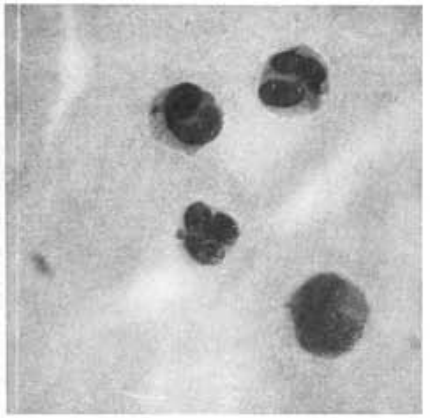

Fig. 6.

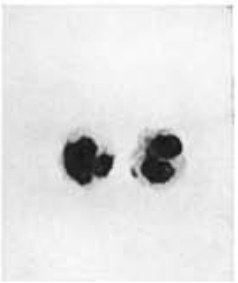

Fig. 7.

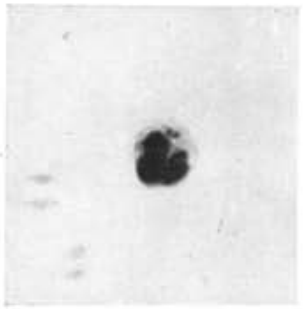

Fig. 8.

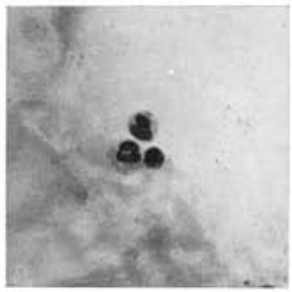

Fig. 9.