

THE ETIOLOGY OF TYPHUS FEVER.

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CONTENTS.

	PAGE
I. The microscopic examination of the blood	156
Summary	160
II. The bacteriological examination of the blood, cerebro-spinal fluid, etc.	160
The characters of the diplococci isolated	161
Opsonins and agglutinins	162
Summary	166
III. The virus	166
IV. The examination of the urine and faeces	168
V. The differentiation of typhus from typhoid fever.	169
VI. The origin of typhus fever and the manner in which it is spread	172
Conclusions	173
References	174

TYPHUS FEVER is a disease which modern sanitary reform has banished from the midst of progressive communities. As a result few opportunities are now afforded for investigating the disease by the bacteriological methods of to-day. Sporadic cases and small epidemics occur from time to time in the few insanitary districts that remain in Belfast. Dr Ernest H. Milligan and I made some observations of a bacteriological nature on this disease which we communicated to the Ulster Medical Society in 1908. Since that time I have continued the investigations when any opportunity occurred and in this paper are recorded the results which have been obtained. Recently some new light has been thrown on the etiology of typhus fever, so the present time appeared to me to be opportune to publish our own observations, to analyse the findings of other observers and to discover what is truly established with regard to this subject.

I have divided the paper into sections in each of which I first relate the observations made in Belfast, then those of other investigators and finally I state the conclusions which I think can be fairly drawn in the present state of our knowledge.

I.

The microscopic examination of the blood.

Blood films were made from 12 patients at various stages of the fever. These were at once fixed in absolute alcohol and afterwards coloured by Leishman and Giemsa's dyes, methylene blue, dilute carbol-fuchsin and thionin blue. The stains were allowed to act from 5 minutes to 24 hours. A careful search for protozoon parasites was made but only on one occasion was a body found which bore any resemblance to such organisms. This structure, which was ovate in shape, measured about $6\ \mu$ in length and $1.5\ \mu$ in breadth and contained two little round deep red-stained dots (Giemsa's stain was used), one being centrally situated the other at one of the poles. It was very similar in appearance to Fig. 6 of the bodies described by Krompecher, Goldzieher and Augyán (1909).

The most striking feature of the blood films was the great number of large mononuclear cells and the absence of eosinophile cells. I made a differential count of several thousand white cells in films taken at different stages of the disease in 12 different patients. The following is a brief summary of my findings. In no case was an estimation of the total number of white cells present determined but there was evidently a leucocytosis in most of the cases.

Polymorphonuclear leucocytes. During the febrile period the relative proportion of polymorphonuclear cells varied from 50 to 80%. On an average 70% of the cells belonged to this class.

Large mononuclear cells of Ehrlich. In this group we include hyaline and transitional cells. A relative increase of these cells was one of the most striking features of the films. The nuclei of these cells were round, oval or reniform and the cytoplasm was abundant. Vacuoles were sometimes seen in the cytoplasm and on one occasion in the nucleus itself.

Occasionally large cells were seen with two nuclei, giving one the impression that the cell was in the act of division. The large mononuclear cells increased in relative proportion as the disease advanced, and when the temperature dropped to normal they formed on an average

25 % of the leucocytes. The increase of mononuclear cells persisted during convalescence; patients whose temperature had been normal for four weeks had about 20 % of their leucocytes of this type.

Lymphocytes. The lymphocytes large and small were relatively reduced, their place apparently being taken by the large mononuclear cells. On an average the combined large and small lymphocytes amounted to 10 % of the leucocytes.

Eosinophiles. During the acute stage of the disease these were absent or present in very scanty numbers. In 3755 leucocytes counted during the febrile period only 8 were eosinophiles, a proportion of .2 %. With the defervescence of the fever their numbers rose and soon they regained their normal proportion. In two cases we observed a subsequent eosinophilia during convalescence, the eosinophile cells forming in these cases 12.8 % and 16.5 % respectively of the leucocytes.

Myelocytes. An occasional myelocyte was found in some of the films.

Having thus given the results of my own examination I shall now refer to those recorded by other observers.

Mott and Blore (1883) in examining drops of fresh blood taken from 12 cases of Typhus Fever saw dumb-bell shaped bodies which they regarded as micrococci undergoing division. Single cocci 5μ in diameter were also seen. Cultures were not made. They noted that the white blood corpuscles were increased.

Thoinot and Calmette (1891) found a leucocytosis present in the blood of four Typhus cases examined. In the blood withdrawn by splenic puncture in four cases and in that obtained from the finger in a fifth case they saw little refractile bodies 1μ to 2μ in diameter provided with a short cilium and moving with great rapidity between the red cells. When the same drop of blood was examined 12 to 24 hours later the little bodies were not visible but instead refractile threads 10μ to 30μ in length were seen displaying a serpentlike motion amid the blood cells. These filaments had at one end a round or oval swelling equal to $\frac{1}{3}$ or $\frac{1}{4}$ the diameter of an erythrocyte. Thoinot and Calmette were unable to obtain any cultures. They recognised that bodies similar to those they described had been observed in other diseases so that they were unable to decide whether these structures represented stages in the breaking down of blood corpuscles or whether they were specific elements.

Soon after Lewaschew (1892) described structures in fresh blood which very closely resembled blood platelets but differed in having a smaller volume, a more regular outline, greater and more spontaneous

motility and also in the possession of a thread-like process. In short the figures and descriptions of the bodies of Thoinot and Calmette and those of Lewaschew are very similar. Lewaschew obtained cultures of cocci which varied from $2\ \mu$ to $5\ \mu$ in diameter and which had often long cilia. He believed that these cocci represented a stage in the life-cycle of the bodies which he saw in the fresh blood as is evident from the following quotation: "although one usually in the investigation of Typhus blood finds three forms of micro-organisms—cocci, cocci with threads attached, and free threads (spirochaetae), it is very probable that these represent only different forms of one micro-organism."

Benjasch (1899) and Stanichevskaja (1905) were able to confirm the discovery of Lewaschew, whilst Weinschal (1892) in 10 cases obtained entirely negative results both as regards microscopic and cultural examination of the blood.

Gotschlich (1903) described in the blood of Typhus cases parasites which in his opinion stood nearly related to *Piroplasma (Babesia) bovis*. His descriptions of the bodies are as follows: (1) Endoglobular pear-shaped parasites varying in diameter from $1\ \mu$ to $4\ \mu$. These were observed in six cases. (2) Flagellated bodies like spermatozoa consisting of an oval body $1.5\ \mu$ in its long axis and provided with a long winding flagellum. These bodies were actively motile and were very similar in appearance to those described by Thoinot and Calmette and Lewaschew. (3) Cysts of an oval or round form and about the size of erythrocytes. These contained 3—6 intensely staining round bodies at the edge and were regarded by Gotschlich as sporulation forms. They were found in only a single case. Kireef (1905) and Horiuchi (1908) were unable to confirm Gotschlich's observations, as in their hands microscopic and cultural examination of Typhus blood gave negative results.

Love (1905) concluded (1) that Typhus Fever is always accompanied by a leucocytosis. The average number of leucocytes in a series of 26 cases was found to be 24,000, the numbers ranging between a minimum of 8000 and a maximum of 54,000 per cubic mm.; (2) that the character of the leucocytosis is practically the same in all cases and corresponds with that found in certain of the other exanthemata, e.g. scarlet fever, inasmuch as it results mainly in an increase of polymorphonuclear cells; (3) that in the blood of fatal cases there are no eosinophile cells whilst in non-fatal cases these corpuscles are always present; (4) in non-fatal cases there is occasionally a slight relative increase in the large mononuclear elements; (5) that the red corpuscles are usually increased in numbers.

Love contrasted the leucocytosis of Typhus Fever with the leucopenia of Typhoid Fever and in his opinion a blood examination would be sufficient to distinguish the two diseases "though," he admitted, "there is some apparent similarity in the manner in which the large mononuclear and eosinophile cells behave." The character of the blood in Typhus suggested to him that the disease was due to a diplococcus since it was very similar to that found in Pneumonia and Rheumatic Fever. Love was unable to confirm Gotschlich's opinion as to the presence of protozoa in the red blood corpuscles and although he met with bodies somewhat similar to those described by Gotschlich he regarded them as portions of the corpuscles that had undergone degenerative changes.

Slatinéano and Galesesco (1906) made a cytological examination of the blood of 16 cases. They concluded their paper with these words: "In Typhus Fever there is a polymorphonuclear leucocytosis and the variations in this are explained by the fact that in the course of the disease there are produced a series of secondary infections. But what strikes the attention is the enormous and constant increase of the mononuclears which is attained at the end of the disease. They may amount to 45% of the leucocytes. It may be supposed that this increase of mononuclears represents the reaction of the body against the unknown parasite."

On examining a drop of fresh blood they found in most cases a little dumb-bell shaped corpuscle 2—3 μ in length and having the poles more refractile than the central part. It exhibited slight movements of oscillation but not of translation. This little corpuscle was sometimes phagocytosed by the mononuclears and lay in a vacuole within them. Vital staining by neutral red and methylene blue showed two polar points more deeply stained than the central part. They never found the body in preparations stained in the usual way.

Lucksch (1907) examined the blood of 21 cases and found with but few exceptions a leucocytosis present. The eosinophiles were absent and the mononuclear cells were increased. No micro-organisms were found in the blood by cultural or microscopic examination. Krompecher, Goldzieher and Augyán (1909) in blood films made from 48 cases of Typhus Fever and stained by Giemsa's method saw structures which in their morphological and staining characters as well as in their position inside the red blood corpuscles resembled at one time malarial parasites at another piroplasmata. They considered the possibility of the bodies being degenerated portions of corpuscles but rejected this interpretation and concluded that they were protozoa.

SUMMARY OF SECTION I.

From these various observations we are justified in drawing the following conclusions:

(1) That in Typhus Fever there is generally a leucocytosis (Mott and Blore, Love, Slatinéano and Galesesco, Lucksch).

(2) That the polymorphonuclear leucocytes are increased but that the most striking feature is the increase of the large mononuclear cells and the absence of eosinophiles (Slatinéano and Galesesco, Lucksch, Wilson and Milligan).

(3) That a post-febrile eosinophilia is sometimes observed (Wilson and Milligan).

(4) That it must be left an open question whether the flagellated bodies described by Thoinot and Calmette and Lewaschew are parasites or breaking-down cells though on the whole the evidence points to the latter conclusion.

(5) That the dumb-bell shaped bodies described by Mott and Blore and by Slatinéano and Galesesco in my opinion represent micrococci in the act of division.

(6) That the protozoon nature of the bodies described by Gotschlich, Krompecher, Goldzieher and Augyán and myself has not been established.

II.

**The bacteriological examination of the blood,
cerebro-spinal fluid, organs, &c.**

Our routine procedure was to withdraw aseptically from the median basilic vein 5—10 c.c. of blood and to introduce it into a flask containing 100 c.c. of bouillon. The flask was then incubated at 37° C. for 24—48 hours and subcultures made on solid media. Occasionally we used glucose bouillon and several times the blood was received in melted agar at a temperature of 43° C. For subculturing from the flasks the solid media used were agar, ascitic-agar, glucose-ascitic-agar. In a few cases the blood was mixed with an equal quantity of 1% potassium citrate solution and then incubated at room and body temperature. It was only when we introduced the blood into bouillon that we succeeded in obtaining cultures.

Thirty-three cases were examined, in 18 instances with negative results whilst in 15 cultures of diplococci were obtained. In most cases the examination was made about the end of the first or early in the second week of the illness.

The characters of the diplococci isolated.

Morphology. Small gram-positive diplococci: occasionally short chains consisting of four or six individuals were seen. Growth on agar and gelatin—greyish film, bluish by transmitted light. No formation of pigment. Growth rather like a culture of the *B. typhosus*, quite unlike a culture of *Staphylococcus aureus* or *albus*. In about half the cases liquefaction of the gelatin commenced about the tenth day whilst in the others none was evident even after six weeks' cultivation. In bouillon a uniform turbidity was produced. In one case an ovoid gram-positive diplococcus or streptobacillus was isolated. This differed from the others in showing a more delicate film of growth and in the rapidity with which it fermented raffinose and clotted milk. The fermentative activity of these cocci was investigated by growing them in litmus broth containing 1% of certain sugars, alcohols, glucosides, and aldehydes. The results obtained with two strains of the diplococci and with the streptobacillus are seen in Table I.

TABLE I.

	Glucose	Laevulose	Galactose	Maltose	Saccharose	Lactose	Glycerine	Mannite	Dextrin	Sorbit	Rhamnose	Xylose
Diplococcus No. 1	+	+	+	+	+	+	+	+	+	-	-	-
" " 2	+	+	+	+	+	+	+	-	+	-	-	-
Streptobacillus	+	+	+	+	+	+	-	-	+	-	-	-

	Raffinose	Arabinose	Adonite	Dulcite	Saltin	Amygdalin	Inulin	Litmus milk	Gelatin
Diplococcus No. 1	-	-	-	-	-	-	-	clot	no liquefaction
" " 2	-	-	-	-	-	-	-	clot	slow "
Streptobacillus	+	-	-	-	-	-	-	clot	no "

The sign + denotes formation of acid; - indicates no change of reaction.

It will be seen that the two strains of diplococci differ from each other in that the one ferments mannite and does not liquefy gelatin whilst the other ferments mannite but liquefies gelatin slowly.

From different members of the same family we have on several occasions obtained cultures of diplococci which in all their biological characters were identical.

Pathogenicity. Mice and guinea-pigs inoculated subcutaneously suffered no ill effects.

On only one occasion had we an opportunity to make a post-mortem examination. In this case smears from the spleen on agar gave no growth but a little piece of spleen added to glucose-ascitic bouillon and incubated afforded a growth of cocci which in all their characters resembled diplococcus No. 2.

Lumbar puncture was performed in one case and the cerebro-spinal fluid was found to be sterile.

Opsonins and Agglutinins.

The opsonic index of the serum of four cases was tested from time to time with reference to diplococcus No. 1. The index was found to vary from .5 to 1.9, rising as convalescence approached. Though we do not lay much stress on our results still they support the view that the cocci had actually been obtained from the patients' blood and were not mere contaminations from the skin. Agglutination experiments afforded strong confirmatory evidence of this view.

In testing for agglutinins emulsions in normal salt solution of a 24 hours' agar culture of diplococcus No. 1 and diplococcus No. 2 were used. Such emulsions had no tendency to spontaneous clump formation. Diplococcus No. 2 was agglutinated in slightly higher dilutions than diplococcus No. 1 both by normal and by typhus serum. The results were recorded at the end of two hours at room temperature.

We tested the action of the blood serum of 13 different cases of Typhus Fever and found that it had from 5 to 20 times the agglutinative effect of normal serum. In all cases we made control tests with the blood of healthy men. An example of an agglutination experiment is given in Table II.

In the case of six healthy adults a dilution of 1 in 20 was the limit at which agglutination occurred. In a few cases of typhoid fever and cerebro-spinal fever we got agglutination in dilutions of 1 in 100. This result certainly impugned the value of the reaction but we must remember that in these diseases heterologous agglutination is a frequent phenomenon.

TABLE II.

Agglutination of Diplococcus No. 1 isolated from the blood of case No. 1.

	Dilutions employed							
	1 in 20	50	100	200	300	400	500	600
Typhus patient's serum No. 1	+++	+++	+++	+++	+	+	+	-
" " " No. 2	+++	+++	+++	++	+	-	-	-
" " " No. 3	+++	+++	+++	+	+	-	-	-
Controls:								
Appendicitis case ...	+	-	-	-	-	-	-	-
Normal blood serum No. 1	+	-	-	-	-	-	-	-
" " " No. 2	+	-	-	-	-	-	-	-

In this and the following tables +++ indicates marked, ++ moderate, + slight agglutination; - absence of agglutination.

These agglutinins could be removed by saturating the serum with the coccus. One drop of the serum was taken and 19 drops of normal salt solution added to it. To this several loopfuls of the growth from an agar culture of the coccus were added. After four hours the mixture was centrifuged and it was then found that the supernatant serum had lost its agglutinative action on the coccus. In Table III details of such an experiment are given.

TABLE III.

	40	60	100	200	300	400
Serum before saturation	+++	+++	+++	+++	+	+
" after "	-	-	-	-	-	-

An experiment was devised to determine whether the agglutinins acting on diplococcus No. 1 and on diplococcus No. 2 were the same or distinct. Such an experiment is shown in Table IV. The serum was that of a patient in the ninth day of the disease.

TABLE IV.

Titre of the original serum.	Dilutions employed						
	40	60	100	200	300	400	500
Diplococcus No. 1	+++	+++	++	+	+	-	-
" " 2	+++	+++	+++	+++	++	+	-
Titre of serum which had been saturated with diplococcus No. 1 for 16 hours at room temp.							
Diplococcus No. 1	-	-	-	-	-	-	-
" " 2	+++	+++	++	+			
Titre of serum which had been saturated with diplococcus No. 2 for 16 hours at room temp.							
Diplococcus No. 1	+	-	-	-	-	-	-
" " 2	-	-	-	-	-	-	-

This experiment shows that saturation with diplococcus No. 2 removes all its own agglutinins and most of the agglutinins that act on No. 1. Saturation with diplococcus No. 1 removes all its own agglutinins but only half of the agglutinins that act on diplococcus No. 2.

In a paper published in 1893 A. Gouget gave a summary of the bacteriological investigations that had been made up to that date with regard to Typhus Fever. We quote the following passage from that paper: "In 1881 Brautlecht isolated from the urine of several Typhus Fever patients a microbe which he considered characteristic but which has never since been found. The same objection applies to the micrococcus observed by Hallier in the blood and sought for in vain by Rosenstein as well as to the bacillus described by Moreau and Cochez. Mosler found the blood of several Typhus cases taken during life sterile and like Obermeier he had negative results when he inoculated animals with such blood. Zülzer in the course of his animal-inoculation experiments obtained one positive result but doubt is cast on its specific nature by Eichhorst. Obermeier obtained no result on inserting the blood of a Typhus case under the epidermis of a healthy man."

Hlava (1888) obtained cultures of gram-positive streptobacilli from the blood. On agar, serum and in bouillon good growth occurred whilst there was no growth on gelatin and on potato. The cultures were not pathogenic for the usual laboratory animals but two young pigs when inoculated presented febrile symptoms lasting a fortnight and the one which had been inoculated in the lung presented reddish blotches on its skin. Hlava also obtained cultures of staphylococci and pneumobacilli.

We have already referred to the fact that Mott and Blore (1883) saw in drops of fresh blood bodies that they regarded as diplococci and that Lewaschew (1892) obtained cultures of micrococci. Calmette (1893) obtained from the blood, sputum and urine of six cases of Typhus Fever cultures in acid media of a fungus which belonged to the class of Ascomycetes or Ustilagineae; he believed that the bodies previously seen by him and Thoinot in the microscopic examination of the blood represented a stage in the life-history of this organism.

Dubief and Bruhl (1894) examined bacteriologically nine cases of Typhus Fever and on six of these they made a post-mortem examination. Cultures were difficult to obtain from the blood though diplococci could be seen on microscopic examination, but from the sputum and from the pneumonic patches in the lungs cultures were readily obtained. The diplococcus named by its discoverers *Diplococcus exanthematicus* grew well on the ordinary media and liquefied gelatin. On agar the

growth appeared as a greyish streak at the end of 24 hours, at the end of 48 hours it became yellowish but finally assumed its former colour. Animal inoculation did not yield satisfactory results.

Curtis and Combemale (1893) were unable to obtain cultures from the blood of patients during life but from the spleen and brain of three fatal cases they obtained cultures of diplococci which very closely resembled those described by Dubief and Bruhl. Weinschal (1892) in 10 cases, Fuchs (1896) in 7 cases, Spillmann (1896) in 5 cases, McWeeney (1898) in 2 cases failed to obtain any cultures of micro-organisms.

Balfour and Porter (1899) from drops of blood obtained cultures of gram-positive diplococci in 36 out of 43 cases examined. Control examination of the blood of healthy individuals yielded negative results but a similar organism was obtained in 40 out of 46 cases of Typhoid Fever. They described the growth on agar as similar in appearance to a streak of white cement, copious and rapid in its spread. Liquefaction of the gelatin began about the fourth day.

Stanichevskaja (1906) examined by cultural methods the blood of 19 cases of Typhus Fever. Cultures were obtained only when large quantities (4—5 c.c.) of blood were withdrawn into 100 c.c. of broth. In 11 cases the flasks remained sterile, whilst in 6 cultures of diplococci were obtained which from the description were probably identical with those isolated in Belfast. Of the 2 remaining cases one gave a growth of a diplobacillus and the other of a bacterium.

Kireef (1905) in 12 cases examined obtained negative results in 9 and in the remaining 3 where cultures of streptococci were obtained he attributed the growth to a contamination.

Galesesco and Slatinéano (1906) found the blood in 18 out of 24 cases sterile. On four occasions a gram-positive diplococcus and on six a gram-negative non-motile bacillus was discovered. This same bacillus was found in the cerebro-spinal fluid of eight out of 24 cases examined. In five cases an autopsy was made; the heart's blood of four gave a culture of gram-positive diplobacilli and of one the gram-negative bacillus with a streptococcus. The cerebro-spinal fluid yielded in four cases out of the five the gram-negative bacillus accompanied by pneumococci. The gram-negative bacillus possessed the following characters. It was non-motile, reddened the Drigalski medium, fermented lactose with formation of acid but no gas, coagulated milk in 48 hours, formed indol in 24 hours and did not liquefy gelatin. It was slowly agglutinated in dilutions of 1:50 and 1:100 by the blood serum of Typhus patients.

Galesesco and Slatinéano regarded these micro-organisms as secondary invaders and believed that the increase of the large mononuclear cells in the blood pointed to the pathogenic agent being a protozoon.

Krompecher, Goldzieher and Augyán (1909) found in the blood streptococci, staphylococci and pneumobacilli which they regarded as secondary invaders and which probably aggravated the clinical condition.

Rabinowitch (1909) in sections by special methods of staining demonstrated gram-positive bacilli and also obtained cultures of the same.

SUMMARY OF SECTION II.

Little weight can be attached to the result of blood-culture experiments performed before 1900 as prior to that date in most cases only drops of blood were taken or the results were based on the examination of too small a number of cases. The results of modern investigators (*e.g.* Stanichevskaja, Galesesco and Slatinéano, Wilson and Milligan) show that gram-positive diplococci can in a large proportion of cases be cultivated from the blood. Our agglutination experiments tend to show that such organisms were actually infecting the persons from whom they were isolated. Whether the diplococci described by various observers should be regarded as identical is very doubtful. The diplococci of Dubief and Bruhl formed pigment, and those of Balfour and Porter produced a growth "resembling a streak of cement," descriptions which suggest that these observers were dealing with cultures of the *Staphylococcus aureus* and *Staphylococcus albus* respectively. We believe that the organisms described by Stanichevskaja and by ourselves are identical. In our cases the cultures on agar were semi-transparent and there was a complete absence of the pigment formation and opaqueness characteristic of cultures of staphylococci. Whether these diplococci are to be regarded as the causative organisms or merely as secondary invaders must be left undecided at present: recent investigation supports the second hypothesis. With this we shall now deal in Section III.

III.

The Virus.

Mochutkovski (1900) after seven unsuccessful attempts at last succeeded in infecting himself by inoculation with the blood of a Typhus Fever case. The incubation period was 17 days.

Yersin and Vassal (1908) succeeded in conveying Typhus Fever to two natives of Indo-China by subcutaneous injection of .5 c.c. of blood taken from patients in the second and fifth day of the disease. The incubation period in the first case lasted 14 and in the second 21 days. Yersin and Vassal could discover no signs of the virus in the blood but like Galesesco and Slatinéano and ourselves they found towards convalescence a characteristic increase of the large mononuclear cells.

Nicolle (1909) transmitted Typhus Fever from a patient to a chimpanzee by inoculating the latter with 1 c.c. of the patient's blood. From the chimpanzee the infection was conveyed to a monkey (*Macacus sinicus*) although direct inoculating of such an animal with the blood of an infected man gave negative results. Nicolle's experiments proved the presence of the virus in the blood of man on the day on which the eruption first appeared and in the blood of the chimpanzee two days before its appearance.

Anderson and Goldberger (1910) conveyed to two species of monkeys, *Macacus rhesus* and *Cebus capuchinus*, a characteristic fever by inoculation with the blood of Typhus Fever cases. After an incubation period of 5—11 days the fever continued for 13 days and in its onset, course, duration and critical defervescence presented a striking resemblance to the fever curve of this disease in man. The eruption was absent. The human blood with which they inoculated gave no visible growth on ordinary culture media. Passage experiments supported the view that they were dealing with a living virus capable of multiplication. The virus was apparently too large to pass through a Berkefeld filter since the authors found that diluted defibrinated human blood after filtration when inoculated into monkeys gave no febrile reaction or other manifestations of illness.

Ricketts and Wilder (1910) in this and other points independently obtained results similar to those of Anderson and Goldberger. These experiments of Anderson and Goldberger strongly support the view that the micro-organisms described by various observers should be regarded as only secondary invaders and that the virus cannot be grown on the ordinary media. We may note however that we have seen what appeared to be diplococci in the blood of some of the cases from which we were unable to obtain cultures.

IV.

Examination of the urine and faeces.

On two occasions we cultivated from the urine of two different patients bacilli which only differed from the *B. coli communis* in that they failed to ferment lactose. These bacilli were agglutinated in dilutions of 1:50 and 1:100 by the patients' serum whilst normal serum had no effect.

The faeces of seven cases were examined, the Conradi-Drigalski and Fawcus' modification of Conradi's Brilliant Green medium being employed. The blue or clear colonies were tested as regards agglutination with the serum of a typhus fever case. In only one case did agglutination occur. In this case there were more blue colonies than red ones on the Conradi-Drigalski plate. The bacilli composing the blue colonies were agglutinated in high dilutions by the blood serum. This bacillus in cultural characters differed from the *B. coli communis* in that its fermentation of lactose was very slow. In a previous paper we gave details of the agglutinative action of the blood serum of Typhus Fever cases on this bacillus which for convenience of description we shall refer to as the Bacillus U. The serum of 17 cases of Typhus Fever examined had on an average from 3 to 10 times the agglutinative effect of normal serum. The agglutinins could be removed from the serum by saturating it with the Bacillus U. but this saturation did not remove the agglutinins for the typhoid bacillus where such were present, neither did saturation with the typhoid bacillus affect the agglutinins for the Bacillus U. The following experiment proved this.

TABLE V.

	Dilutions									
	40	60	200	400	600	800	1000	1200	1400	1600
Untreated serum of patient F. M ^c C.										
Agglutination of Bacillus U.	+++	+++	+++	+++	+++	+++	+++	+++	++	+
Agglutination of Typhoid b.	+	+								
Serum saturated with Bacillus U.										
Agglutination of Bacillus U.	-	-	-	-	-	-	-	-	-	-
Agglutination of Typhoid b.	+	+								
Serum saturated with typhoid bacillus.										
Agglutination of Bacillus U.	+++	+++	+++	+++	+++	+++	+++	+++	++	+
Agglutination of Typhoid b.	-	-	-	-	-	-	-	-	-	-

We may add that the agglutinins which acted on the diplococci and which acted on the *B. typhosus* and on the Bacillus U. were proved by saturation experiments to be distinct.

Horiuchi (1908), in a fever clinically resembling Typhus Fever, obtained from the stools and in 3 cases from the urine of 40 cases examined a bacillus which we found to be culturally identical with the Bacillus U. Horiuchi believed that the fever in question was due to his bacillus and the fact that some of the cases agglutinated the *Bacillus typhosus* also he attributed to the presence of group agglutinins. The bacillus was never obtained from the blood.

The fact that intestinal organisms should be found by different observers to be agglutinated by the blood serum of Typhus Fever cases is interesting but what its significance is cannot be at present determined. In Section V we shall refer to this matter in greater detail.

V.

Differentiation of Typhus from Typhoid Fever.

The fact that it was not until the fourth and fifth decades of the nineteenth century that these diseases were recognised as two separate entities shows how closely akin the older physicians imagined them to be. Those who have much experience of Typhus Fever know that there are cases in which it is impossible to make a clinical distinction. Ker refers to such cases when he states "but even if we fail to make the distinction clinically we have always the Widal test at our disposal." I shall presently show that the Widal test affords but little assistance.

Many writers separate Typhus Fever from Typhoid Fever and classify it with the acute exanthemata (Small Pox, Scarlet Fever, Measles) which it resembles in its infectivity, onset and rash; on the other hand it differs entirely from these in the fact that it is always associated with unhygienic conditions whilst the other diseases named have never been shown to be influenced as to their incidence by good or bad sanitation.

We have already referred to the fact that in Typhoid Fever a leucopenia and in Typhus Fever a leucocytosis is the rule. However in many respects there is a resemblance in the blood picture presented by Typhus and Typhoid Fever. Thayer (1902) states that the three main changes in the blood in Typhoid Fever are (1) a progressive diminution

in the percentage of the polymorphonuclears, (2) a progressive increase in the percentage of mononuclear forms, the increase being mainly in the large mononuclear varieties, (3) a constantly small percentage of eosinophiles. We have noted that all these changes occur in the blood of Typhus cases. There is no doubt that in Typhoid and Typhus Fever the blood is characterised by an increased proportion of large mononuclear cells. We may attribute this reaction to the view that the causative organism is a protozoon; or on the other hand may it not be due to the fact that in both diseases there is an infection with organisms of the coli-typhoid group? We believe that our experiments and those of Horiuchi prove that in Typhus Fever such an infection (secondary though it probably is) does occur. Such infecting organisms in Typhus however do not enter the blood stream though they may be found in specimens of urine taken with every aseptic precaution. They probably reach the urine through the lymphatic system. We may here recall Coleman and Buxton's (1909) view with regard to the pathogenesis of Typhoid Fever. These observers believe that in this disease the atrium of infection is the lymphatic structures of the intestinal wall whence the bacilli invade the general lymphatic system and spleen and from these systems the blood is only secondarily invaded.

As regards the value of the Widal test in assisting in the differential diagnosis the following is our experience. The blood serum of 35 cases of Typhus Fever was tested in reference to its agglutination of *B. typhosus*. In 19 instances the reaction was positive; in 16 it was negative where a 1 in 50 dilution of the serum was used. In several cases higher dilutions were employed: in many of these good clumping was obtained in 1 hour in a 1 in 100, in several in a 1 in 200 and in one in a 1 in 300 dilution. On several occasions different strains of typhoid bacilli were employed and almost invariably our results were controlled by the examination of non-typhus blood. I may add that the patients from whom the blood was taken were undoubtedly suffering from Typhus Fever. The clinical picture was typical and in nearly all cases many members of the same family were attacked. Moreover the blood was examined by cultural methods in 33 cases and the flasks either remained sterile or there was a growth of diplococci but never of bacilli. We may conclude that the Widal test for the purposes of diagnosis is of little value in this connection. Patterson (1908), from observations in Lanarkshire cases, independently reached the same conclusion.

Patterson made 38 Widal examinations of the blood of 10 cases of Typhus Fever, employing seven different strains of typhoid bacilli. In nine cases a positive and in one a negative reaction was obtained. The dilutions of the serum employed ranged from 1:50 to 1:1000 and in four cases a positive result was obtained at some stage of the disease even with the latter dilution. The faeces, urine and blood were culturally examined for the *B. typhosus* but with negative results. Patterson found that the reaction was obtained as early as the ninth day of the illness, was most marked about the fourth week and gradually passed off about the sixth week.

Patterson's results and ours differ from those recorded by most writers on this question as is evident from the following quotations:— "After trying the Widal reaction in nearly a hundred cases of Typhus Fever I have only found it positive twice" (Ker, 1909). "The serum of a patient suffering from typhus does not clump typhoid bacilli" (Goodall and Washbourn, 1908). "The Gruber-Widal agglutination test will enable us in many cases to differentiate the two diseases" (Curschmann, 1902). "The agglutination test will establish the diagnosis of enteric fever" (Moore, 1906). "The Widal reaction and blood cultures are important aids" (Osler, 1909). Love (1905) mentions that in Typhus Fever a positive Widal reaction is sometimes obtained. Iversen (1905) obtained a negative Widal with 10 cases of Typhus Fever whose blood he tested. Galesesco and Slatinéano (1906) found that the serum of their patients never agglutinated Eberth's bacillus, *B. coli communis* or paratyphoid bacilli.

The results obtained by Horiuchi, Patterson and ourselves definitely prove that in Typhus Fever agglutinins for the typho-coli group of micro-organisms are present in the blood serum of the patients, but the knowledge which has recently been acquired with regard to the presence of heterologous agglutinins in cerebro-spinal fever prevents us from drawing the unwarranted conclusion that the presence of a bacillus in the intestine and urine and the discovery of agglutinins for it in the blood indicate that such an organism is the cause of the disease in question. Though future research may show that the causative organism of Typhus Fever is in no way related to diplococci or to any variety of intestinal organism still the presence of agglutinins for these organisms in the serum probably indicates that the latter are infecting the patient. We are now beginning to learn that the body in infection has not only to deal with the specific microbe and its toxins but also

with certain organisms contained in the alimentary canal which are normally saprophytic but which in the altered conditions of metabolism produced by disease become to some degree pathogenic.

VI.

The origin of Typhus Fever and the manner in which it is spread.

All observers agree that Typhus Fever is a disease which is always associated with filth, overcrowding and privation. Some observers, such as Murchison and Griessinger, maintained that these conditions were sufficient to cause the disease to arise spontaneously. When a case of the disease is once established the cases following can be shown in nearly all instances to have been exposed to infection direct or indirect of other cases. In one of the Belfast cases the virus appeared to have been carried in a shawl. In Belfast the infection could as a rule be traced to previous cases but "a number remained in which (to quote the words of Dr Bailie, M.O.H. Belfast, 1908) it was quite impossible to trace the infection, so obscure were they that one was almost compelled to give consideration to the old hypothesis of Murchison, *i.e.* spontaneous or *de novo* origin of Typhus Fever." Osler (1909) refers to outbreaks in which the source of infection could not be found.

The recently established facts regarding "Typhoid carriers" have served to clear up the origin of many outbreaks of enteric fever which in former times would have been taken as presenting clear evidence for the *de novo* theory. Kelsch (1893) believed that tramps might convey Typhus Fever although themselves healthy. Newsholme (1908) has shown from the Irish figures the close relationship that exists between Typhus Fever and poverty; vagrancy in his opinion was the great factor in the dissemination of the disease.

How poverty, overcrowding and bad sanitation cause Typhus Fever may be explained in three ways: (1) that these factors diminish the resisting power of the body to the action of a specific virus which is not generated by the conditions; (2) that these conditions may lead to the development of the virus either in the environment of the first person affected or in his alimentary canal. Such an unhygienic mode of life might lead to an alteration of the flora of the person's alimentary canal with the resulting possibility of certain germs acquiring the power of infecting the patient. Kelsch is a modern upholder of this hypothesis. In this connection we

may recall the fact that agglutinins for typhoid bacilli and colon bacilli are present in increased amounts in the serum of Typhus Fever cases ; (3) that in such conditions the virus has the necessary means for its propagation, *e.g.* close atmosphere, presence of vermin, etc.

The majority of observers till recently believed that the virus clung to infected objects in the immediate vicinity of the patient and was inhaled entering the body through the respiratory tract.

Chantemesse (1893) and Curschmann (1902) favoured this view. Netter (1893) believed that the virus was swallowed and from the alimentary canal entered the blood : he also mentioned the possibility of the disease being conveyed by the bite of insects. The view that the contagium of Typhus Fever is conveyed from the patient to the healthy by fleas was strongly advocated on epidemiological grounds by Hay (1907).

Nicolle, Comte and Conseil (1909) in Tunis, after passage of the virus through a chimpanzee, were able to infect a monkey (*Macacus sinicus*) and from this to transmit the disease to two other monkeys by means of lice which had fed on the infected monkey and were then allowed to bite the healthy animals. Nicolle considered the possibility of bugs and fleas conveying the disease and came to the conclusion that the incidence of the disease could best be explained by inculcating the body louse. Anderson and Goldberger (1910) in Mexico also found evidence to incriminate body lice as the carriers of infection though their attempts to infect monkeys with Typhus Fever by means of them failed.

CONCLUSIONS.

(1) A relative increase in the large mononuclear leucocytes especially towards convalescence is very characteristic of the blood of Typhus Fever cases.

(2) During the febrile period the eosinophile cells are absent or very scanty in numbers. In two cases an eosinophilia was observed during convalescence.

(3) The blood of 33 different cases was examined by cultural methods. In 18 no growth of micro-organisms occurred whilst in 15 characteristic diplococci were cultivated. These diplococci were agglutinated by the blood serum and the agglutinins could be removed from the serum by saturating it with the cocci.

(4) From the faeces of one case a variant form of *B. coli communis* was cultivated on which the blood serum of 17 Typhus Fever cases was found to have 3 to 10 times the agglutinative effect of normal serum.

(5) From the urine of two cases a bacillus resembling *B. coli communis* but having no action on lactose was cultivated. This bacillus was agglutinated in dilutions of 1:50 and 1:100 by the serum of the cases but not by normal serum.

(6) The facts established by Anderson and Goldberger which showed that the virus of Typhus Fever was present in blood which afforded no growth on ordinary media suggest that the above micro-organisms are secondary invaders.

(7) Of 35 cases examined the blood serum of 19 gave a positive Widal reaction with the *B. typhosus*. Hence this reaction is of little or no value in differentiating Typhus from Typhoid Fever.

(8) The recent work of Anderson and Goldberger, Ricketts and Wilder, Nicolle, Comte and Conseil goes far to prove that the virus is present in the blood and that insects, probably *Pediculi vestimenti*, are the agents by which infection is carried.

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