THE LABORATORY DIAGNOSIS OF TYPHUS FEVER.

FURTHER OBSERVATIONS ON THE VALUE AND ON THE SIGNIFICANCE OF THE WEIL-FELIX REACTION.

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CONTENTS.

Following the capture of Jerusalem and in the early months of 1918 Capt. C. M. Craig, R.A.M.C., obtained a culture of a proteus-like bacillus from the civil Jewish bacteriologist of that city. He was informed that this organism was being extensively used by German and Austrian bacteriologists on the Eastern front for the diagnosis of Typhus Fever. After making some preliminary observations on its agglutination by the sera of typhus patients he forwarded the culture on to this laboratory for further investigation, the preliminary account of which was published by Craig and Fairley (21 Sept., 1918, *Lancet*, pp. 385–6).

In the following pages I propose to put on record a *further series* of personal observations fully supporting the conclusions reached in the above-mentioned report.

1. CONCERNING CERTAIN ETIOLOGICAL ASPECTS OF TYPHUS FEVER.

Clinically, Typhus Fever must be regarded as a septicaemia. The typical course of the disease, the temperature chart, the dark macular or petechial rash, the markedly toxic condition of the patient all combine to impress this conception upon even the casual observer. Furthermore, as Nicolle first demonstrated, the virus of Typhus is present in the peripheral blood during the pyrexial period, for it may then be transmitted directly to certain species of monkeys and to guinea-pigs by subcutaneous injection of whole blood. Under natural conditions, however, the virus is transmitted from man to man by the louse (*Pediculus humanus*)[1]. Ricketts and Wilder maintain that blood is not infectious if passed through a Berkefeld filter, but Nicolle reports that the virus is filterable. Many organisms have been suggested as the causative agent in the production of this disease, especially bacillary, diplobacillary, and diplococcal forms. As recently as 1917 Futuki has described a spirochaete resembling *T. pallidum* as occurring in the kidney and supra-renal glands of patients dying of Typhus. The same organism was found in the kidney of a monkey inoculated with Typhus Fever.

The organism having most claim to pathogenicity appears to be the *B. typhi-exanthematici* isolated by Plotz[2] from the blood of typhus patients in 1915. It is a small pleomorphic Gram positive non-motile bacillus growing anaerobically in ascitic fluid sterile tissue media. The sera of convalescents from typhus, according to Rabinowitch, show both positive complement fixation and agglutination reactions for this organism when used as antigen. Olitsky[3], in his recent immunological studies on this bacillus, has confirmed these serological reactions and has shown the presence in typhus serum of the following specific antibodies, *i.e.* agglutinins, precipitins, immune opsonins, anaphylactic substances and complement-fixing bodies. Numerous other organisms have been described by various investigators until the literature on the etiological aspects of Typhus Fever has become as obscure as it is voluminous. Furthermore despite all past observations on the subject no laboratory test of diagnostic value had been evolved. The recent claims, therefore, of Weil and Felix[4] regarding the diagnostic reliability of their agglutination reaction have become a matter of importance to the clinician and the pathologist alike.

[1] A full account will be found in Nuttall (1917), "The part played by *Pediculus humanus* in the causation of disease." *Parasitology*, vol. x. pp. 44–57, 74–75.

[2] Plotz, Olitsky and Baehr (1915), *Journ. Infect. Dis.* XVII. 1–68.

[3] Olitsky (1917), *Journ. Immunology*, II. 363.

[4] Weil and Felix (1917), *Wien. klin. Wochenschr.* XXX. 393–9; Viteček, *Ibid.* 967–972.

THE WEIL-FELIX REACTION.

In 1915 Weil and Felix, while investigating a group of cases thought to be enterica but giving negative Widal reactions, isolated an organism from the urine which was agglutinated by the patient's serum in a dilution of 1 in 200. The serum of nine other cases, all of which proved to be Typhus Fever, likewise agglutinated this organism which was identified as belonging to the proteus group. This organism was named the X 2. Later the same observers isolated from the urine a second strain of a similar bacillus known as the X 19, which differed from X 2 in being agglutinated in a very much higher titre by the sera of typhus patients (1 in 2000). A number of other German and Austrian workers[1] have reported favourably on the reaction during the past year.

2. CHARACTERISTICS OF ORGANISMS USED IN THE INVESTIGATION.

The organism utilised in the following investigation proved to be a Gram negative slightly motile short bacillus. It grows anaerobically on all ordinary media such as agar and MacConkey's media, and liquefies gelatin. It produces acid and gas in glucose, saccharose, maltose and mannite, but does not ferment dulcite or lactose. It produces indol freely and acid without clot formation in milk. Subcutaneous inoculation with 2 c.c. of a 24 hours' broth culture proved non-lethal to guinea-pigs.

3. AGGLUTINATION PHENOMENA IN TYPHUS FEVER.

Every case in the following series was a typical clinical case of Typhus Fever. In seven cases complications existed at the time the blood was collected for examination. In three cases broncho-pneumonia was present; in another case there was a pelvic cellulitis secondary to perforation of an ulcer of the rectum; in three other cases parotitis had supervened.

The blood picture was investigated in twelve uncomplicated cases. Absence of leucocytosis, or a definite leucopaenia, was the rule during the first week, but this was generally replaced by a moderate grade of leucocytosis during the second week (10,500 to 14,000 per cmm.). The differential count in the second week showed an absolute and relative increase in the polymorphonuclear neutrophile elements and a decrease in the eosinophile cells.

The agglutination was carried out in all cases on a Garrow's agglutinometer[2], the results were read after five minutes rocking. Controls were used in every instance. In a number of cases concomitant observations were made by using the macroscopic method of tube agglutination and incubating at 37° C. for four hours.

With the prevailing temperature conditions of Egypt I have always found the Garrow's agglutinometer a most reliable instrument. Economy of material,

[1] Sterling and Sterling, *Wien. klin. Wochenschr.* XXX. 972–4; Ballner and Finger, *Ibid.* 966–7; Dadez and Kvahelsha (1917), *München. med. Wochenschr.* LXIV. 1379–1381.

[2] Garrow (1917), *Lancet*, I. 262.

the rapidity with which results can be read, and the elimination of the feeble agglutinating power of certain sera upon this organism, are the great advantages of this method.

Using this instrument I regard an agglutination of 1/40 as diagnostic of Typhus Fever. Up to the 6th day of the disease an agglutination in a dilution of 1 in 20 should be regarded as sufficient evidence on which to isolate a case.

Analysis of Cases.

(*a*) Thirty-five cases were examined during the pyrexial period. The titres of the agglutinations were as follows:

Day	Cases	Titre
5th	1	1/40
6th	2	1/80, 1/320
8th	3	1/80, 1/80, 1/1280
9th	3	1/20, 1/40, 1/640
10th	9	1/80, 1/160, 1/160, 1/320, 1/640, 1/640, 1/1280, 1/1280
11th	2	1/40, 1/1280
12th	5	1/20, 1/80, 1/80, 1/640, 1/1280
13th	5	1/640, 1/640, 1/1280, 1/1280, 1/1280

In five febrile cases the exact day of the illness was not known. The titres of the agglutinations obtained were 1/40, 1/320, 1/320, 1/1280, 1/2560.

(*b*) The sera of twenty-five convalescent cases gave positive reactions as follows:

Day of convalescence	Cases	Titre
1st	2	1/160, 1/1280
2nd	2	1/20, 1/1280
3rd	2	1/40, 1/160
4th	3	1/160, 1/160, 1/640
5th	2	1/40, 1/160
6th	4	1/640, 1/640, 1/1280, 1/2560
7th	1	1/1280
8th	1	1/640
9th	2	1/160, 1/640
10th	1	1/160
12th	1	1/320
14th	1	1/1280
15th	1	1/640
16th	1	1/80
22nd	1	1/320

Progressive agglutination readings were made in four cases as follows:

Case 1.	3rd day, negative 1/20	2 days after crisis 1/20 positive
	6th ,, ,, 1/20	6 ,, ,, ,, 1/20 ,,
	8th ,, ,, 1/20	11 ,, ,, ,, 1/80 ,,

This case constituted the mildest clinical type of Typhus Fever of the series.

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Case 2. 5th day, negative 1/20 9th day, positive 1/160
7th „ „ 1/40 11th „ „ 1/1280

This patient died of an overwhelming toxaemia on the 12th day of illness.

Case 3. 6th day, positive 1/320
8th „ „ 1/640
10th „ „ 1/640
12 days after crisis positive 1/160

This patient on the 6th day of illness developed broncho-pneumonia.

Case 4. 6th day, positive 1/80
8th „ „ 1/80
13th „ „ 1/640

This patient recovered.

(*c*) Time incidence in the appearance of agglutinin:

In the series of cases under review the observations made during the first week of the disease are very limited, but in the preliminary report of Capt. C. M. Craig and the writer, the agglutination reactions in twenty-five cases between the 4th and 7th day of the disease were recorded.

These results were as follows:

Day	Cases	Titre
4th	6	1/10, 1/10, 1/40, 1/50, 1/100, 1/1000
5th	10	1/10, 1/10, 1/10, 1/40, 1/50, 1/50, 1/50, 1/50, 1/80, 1/160
6th	6	1/50, 1/50, 1/50, 1/80, 1/100, 1/320
7th	3	1/10, 1/20, 1/320

During the above-mentioned period, though agglutinin was definitely present, its titre was not nearly as high as in the subsequent course of the disease. In the 2nd and 3rd weeks of the disease (*i.e.* in the 2nd week of the febrile period and in the first week of convalescence) the maximum height of the agglutinin curve is attained, as may be ascertained by a perusal of the preceding tables.

A certain proportion of the cases do not develop agglutinin during the course of the fever. In the present series 5 out of 65, or 7·7 % of the cases, failed to agglutinate *B. proteus* in a dilution of 1 in 20 of patient's serum. In four of these cases the agglutinin content was not investigated during convalescence. The remaining case developed agglutinin during the first week of convalescence.

(*d*) The agglutinin content of the patient's blood and its bearing on the prognosis:

There appears to be no direct relationship between the amount of agglutinin present in the circulating blood and the clinical aspect of the case. In the present series the mildest case had no agglutinin for *B. proteus* until the 2nd day of convalescence, whereas some of the most fatal cases had well marked agglutination established as early as the 5th day of fever. Other cases dying later in the disease (11th to 13th day) only developed agglutinin twenty-four hours before death.

(*e*) Control reactions:

The sera of 120 known negative cases have been examined for agglutination against this proteus-like organism.

Of the protozoal diseases, twenty-five cases of syphilis, thirty cases of relapsing fever, and thirty cases of malaria (twenty-five cases of sub-tertian infection, four cases of benign tertian, and one case of quartan) were examined with negative results.

The bacterial infections which included cases of influenza, pneumonia, undulant fever, and enterica group yielded negative results with two exceptions.

In these two cases (with Garrow's agglutinometer) agglutinations in a titre of 1 in 10 were obtained but the reaction failed with higher dilutions.

Recently Captain C. M. Craig, R.A.M.C., informed me he has observed an agglutination of 1/1000 with *B. proteus* in a definite case of typhoid fever, but he was unable to exclude a previous Typhus infection.

In a group of seven cases of Typhus which were tested against stock culture of *B. typhosus* and *B. paratyphosus* A and B, during the pyrexial period, six yielded negative results. In the other case which had been inoculated with T.A.B. nine months previously, *B. paratyphosus* A and B yielded a positive agglutination with the patient's serum in a dilution of 1/40, while *B. typhosus* yielded agglutination in a dilution of 1/1280. At autopsy infection with *B. typhosus* was definitely excluded in this case.

The sera of convalescent Typhus patients tested against stock culture of *B. shiga*, *B. flexner*, Y, *M. melitensis*, *cholera vibrio*, and *B. coli* all yielded negative results. Similarly *B. proteus* failed to be agglutinated by standard immune sera of *B. shiga*, *B. flexner*, *B. typhosus*, *B. paratyphosus* A and B, and of the cholera serum prepared by the Lister Institute.

4. COMPLEMENT-FIXATION REACTION.

Technique employed; preparation of reagents and of antigen from *B. proteus*.

As the sera of typhus patients agglutinated, in a high titre, a saline suspension of this proteus-like organism, an investigation was carried out by means of the complement-fixation method (Bordet and Gengou, 1901, *Ann. Inst. Pasteur*, XV.):

Complement-fixation reactions are dependent on the fact that when antigen, inoculated serum (? immune body) and complement are mixed together immune body firmly combines with antigen and complement in such a manner that complement can no longer be found free in the mixture. If such a mixture is allowed to stand at a suitable temperature, *i.e.* 37° C., for one hour or more, and to it is added a suspension of red blood corpuscles sensitized with a suitable quantity of haemolytic serum, no haemolysis will take place since there is no free or available complement. This constitutes a positive reaction and proves the presence of specific immune body in the inactivated serum.

If the complement is not fixed then haemolysis ensues; this constitutes a negative reaction and demonstrates the absence of specific immune body in the serum under investigation.

The antigens used were in fresh saline (0·85 % NaCl and 0·5 % phenol) suspension prepared from a twenty-four hours' growth of this organism on agar slopes.

The technique employed was similar to that used in the ordinary *quantitative* Wassermann reaction. Three, six, nine and sometimes twelve minimum haemolytic doses of complement were used in the test.

In the first stage of the reaction, quantities of antigen, immune serum and complement were mixed together for one hour at 37° C. Subsequently sensitized sheep's corpuscles were added and final readings were made after another hour's incubation at 37° C.

The results were recorded as follows:

1. P+++++ fixation of 12 M. H. doses of complement
2. P+++ „ 9 „ „
3. P++ „ 6 „ „
4. P+ „ 3 „ „

Antigen. The antigens employed in the present investigation were prepared by two methods:

Antigen A. This antigen consisted of a fresh saline (*v. supra*) suspension of a twenty-four hours' growth of *B. proteus* on agar slants (this antigen was the one used in fifty-eight cases of Typhus Fever, and in animal and human inoculation experiments).

Antigen B. In this method the (fresh) saline suspensions prepared from growths of *B. proteus* on agar slopes (twenty-four hours old), were heated to 56° C. for one hour and then carefully centrifuged. The supernatant suspension was utilised as antigen. (This was used to investigate nine cases of Typical Typhus.) In standardising the antigen it was found advisable never to use more than one-third the anti-complementary dose.

Haemolytic serum was obtained from rabbits by injecting intraperitoneally and intravenously sheep's corpuscles in progressively increasing doses. The serum used in these tests was one of high titre (1/4000). To sensitize the sheep's corpuscles four minimum haemolytic doses of amboceptor or haemolytic serum were used. The M.H.D. of the amboceptor was taken to be that amount of haemolytic serum just sufficient to produce in one hour at 37° C. complete lysis in one volume of a 3 % suspension of sheep's corpuscles with four or five M.H.D.'s of complement.

Sheep's corpuscles. Equal quantities of sheep's blood were mixed with 2 % sodium citrate in physiological saline. Requisite amounts of this mixture received three washings with nine times the volume of physiological saline, and were finally made up to the equivalent of a 3 % suspension of sheep's corpuscles in the same solution.

Sensitization of corpuscles. After four M.H.D.'s of amboceptor had been added, the suspension of corpuscles was placed in the incubator at 37° C. for thirty minutes, and after sensitization kept in the ice-chest till required.

Patient's serum. Blood was usually obtained on the day preceding the test, and kept in the ice-chest till required. The serum was diluted with four times its volume of physiological saline, and heated to 55·5° C. for twenty minutes. Heating in this manner destroys complement and inhibits the anti-complementary properties of certain sera.

Complement. The complement used was obtained from well-nourished guinea-pigs, and collected under sterile conditions. The M.H.D. of complement was always obtained by preliminary titration. The reagents were measured out by means of Donald's dropping pipettes.

The arrangement of the systems for the final tests was as follows:

Row No. 1.	Antigen	1 vol.	+ 2 vols. saline (0·85 %)
	Patient's serum	1 ,,	
	Complement (3 M.H.D.'s)	1 ,,	
Row No. 2.	Antigen	1 ,,	+ 1 vol. saline
	Patient's serum	1 ,,	
	Complement (6 M.H.D.'s)	2 vols.	
Row No. 3.	Antigen	1 vol.	
	Patient's serum	1 ,,	
	Complement (9 M.H.D.'s)	3 vols.	
Row No. 4.	Patient's serum	1 vol.	+ 3 vols. saline
	Diluted complement (3 M.H.D.'s)	1 ,,	

Row No. 4 serves as a serum control and any anti-complementary tendency in each serum examined is thereby demonstrated.

Additional controls used in the test were:

(1) Antigen control, *i.e.* 1 vol. of antigen and 4 vols. of saline (0·85 %).

(2) Antigen 1 vol., pooled negative serum 1 vol., 3 vols. of saline (0·85 %).

(3) Where possible a sure positive serum was included in the series (*i.e.* a monkey inoculated with *B. proteus*).

Analysis of Results.

Complement-fixation reactions in the sera of typhus patients.

In all fifty-eight sera collected from typhus cases in different stages of the disease were examined. Their agglutination reactions were as follows:

In	1	case	the	titre	was 1/2560
,,	13	cases	,,	,,	,, 1/1280
,,	15	,,	,,	,,	,, 1/640
,,	6	,,	,,	,,	,, 1/320
,,	9	,,	,,	,,	,, 1/160
,,	6	,,	,,	,,	,, 1/80
,,	5	,,	,,	,,	,, 1/40
,,	3	,,	,,	,,	,, 1/20

As far as possible blood was collected from the cases during the second week of fever or in the first week of convalescence (*i.e.* 8th–21st days), during that period when the agglutinin content was at a maximum.

In fifty-five out of fifty-eight of the cases examined the complement-fixation reactions were negative. In one case a P+++ reaction was obtained, in another case there was a similar reading, but the control showed the serum to be anti-complementary. In one other case a P++ reaction was recorded.

Examination of non-typhus sera.

Eighty-three sera from non-typhus cases were examined. Of these eighty yielded negative results and three yielded pseudo-positive reactions. One case of rheumatic fever gave a P++++ reaction, and two cases of syphilis yielded a P++ reaction. The sera of twelve other cases of syphilis yielding positive Wassermann reactions were negative.

Investigation of the amount of complement fixed by pooled typhus serum in the presence of B. proteus *as antigen.*

A pooled serum was prepared from nine cases of Typhus all of which yielded high titre agglutinations. The amount of complement fixed by this serum in the presence of *B. proteus* as antigen was found to be identical with the amount fixed by a pooled serum prepared from eleven non-typhus patients. Less than $1\frac{1}{2}$ M.H.D.'s of complement were fixed in each system.

Conclusion. It follows from the above experiments that the formation of agglutinin in the blood of typhus cases for this *particular kind of B. proteus* is not accompanied by the formation of immune body, as indicated by the complement-fixation reaction.

EXPERIMENTS ON ANIMALS.

Ten monkeys were experimented on (seven *Cercopithecus* and three *Macacus rhesus*).

Of these ten monkeys four were used as controls throughout the investigation. The six others were injected sub-cutaneously with one dose of from 1 to 2 c.c. of a twenty-four hours' broth culture of *B. proteus*. In only one of these monkeys was a second injection given and this was to a monkey labelled "Z" at an interval of ten days following the first injection.

In every case, prior to inoculation, the serum of the monkey was investigated for complement fixation and agglutination reactions against this organism. All had negative complement fixation, and all yielded negative agglutination reactions in dilution of one in ten with one exception only. In this case (monkey "W") a positive agglutination of monkey's serum in a titre of one in ten was obtained but the reaction vanished in a higher dilution of 1 in 20. None of the inoculated monkeys showed obvious signs of sickness.

A perusal of Table I will show the serological reactions of monkeys inoculated sub-cutaneously with broth cultures of this organism.

An analysis of the protocol will also show that in all cases a positive complement-fixation reaction was obtained. In five out of the six cases this was present within twelve days of inoculation. In the sixth case (monkey "D") the complement-fixation reaction was negative on the ninth day, though it became positive on the eighteenth day. The agglutination response in this case was not as intense as usual.

In all these cases accompanying the appearance of agglutinin in the blood serum of the inoculated monkeys, immune bodies were produced which had the property of fixing complement in the presence of its specific antigen, namely *B. proteus*.

Inoculation of Man with *B. proteus*.

In order to observe the serological and clinical effects produced by subcutaneous injections of *B. proteus* two of the laboratory staff (R. C. S. and N. H. F.) were inoculated sub-cutaneously with a saline suspension of 3000 million living *B. proteus*. In both cases a systemic reaction followed (generalised aches and pains, headache, fever 100–102° F.); but these symptoms disappeared within forty-eight hours. A local inflammation occurred at the site of inoculation. In one case resolution without suppuration followed, in the other, on the ninth day, the site of inoculation was incised. A small amount of *sterile* pus was present.

Table I.

Serological Reactions in Infected Monkeys.

Days after inoculation	Monkey "Z"		Monkey "W"		Monkey "X"		Monkey "V"		Monkey "D"		Monkey "A"	
	Agglutination	Complement fixation	Agglutination	Complement fixation	Agglutination	Complement fixation	Agglutination	Complement fixation	Agglutination	Complement fixation	Agglutination	Complement fixation
4	1/20	—	—	—	—	—	—	—	—	—	—	—
6	1/160	P++++	1/320	—	—	—	—	—	—	—	—	—
9	1/640	—	1/2560	P++++	1/1280	P++++	—	—	1/150	Neg.	—	—
12	1/1280	P++++	—	—	—	—	1/640	P++++	—	—	1/160	P++
18	1/1280	P++++	—	—	1/640	—	—	—	1/320	P++++	—	—
25	—	—	1/320	P++++	1/320	P++++	—	—	1/320	P++++	—	—
35	1/640	P++++	—	—	—	—	—	—	—	—	—	—
53	1/40	Neg.	1/160	P++	1/160	P++	—	—	1/20	Neg.	—	—

Table II.

Serological Reactions in Man inoculated with living B. proteus.

Days after inoculation	Case 1		Case 2	
	Agglutination	Complement fixation	Agglutination	Complement fixation
Before	Neg.	Neg.	Neg.	Neg.
6th day	1/360	P++	1/160	P+
13th „	1/160	P+	1/640	P++
18th „	1/80	Neg.	1/640	P++

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The result of the serological reactions observed will be found in Table II. In both cases agglutinin and complement fixing bodies appeared in the circulating blood a few days after inoculation.

5. SIGNIFICANCE AND EXPLANATION OF THE WEIL-FELIX REACTION.

Naturally the significance of the Weil-Felix reaction has caused much discussion. The general concensus of opinion as quoted in a leading article in the *British Medical Journal* (12. I. 1918), including that of Weil and Felix themselves, is that *B. proteus* is not the real cause of Typhus, but a specific secondary invader of the body which accompanies the unknown virus of the disease.

That *B. proteus* is not the causative agent in Typhus is indicated by the facts that (1) it cannot be isolated from blood cultures made during the pyrexial period; (2) it is non-pathogenic to man and monkeys when inoculated sub-cutaneously.

The secondary invasion hypothesis, while superficially plausible and while accounting for the presence of high titre agglutinins in the blood serum of typhus cases, is incompatible with the following facts:

(1) Systematic cultural investigations of the blood of typhus cases over different periods of the disease and also of the urine, save in very rare cases[1], yield negative results, even though *B. proteus* grows aerobically and very readily on all the ordinary laboratory media; furthermore it must be remembered that in any case systematic cultural examinations of the urine of any large series of cases will reveal an occasional *B. proteus* infection, hence the cultural findings of Weil and Felix are by no means conclusive.

(2) Serological investigations of typhus sera, while showing the presence of agglutinin for *B. proteus*, have failed to demonstrate the presence of immune body; whereas sub-cutaneous injections into monkeys and man have been followed by the production of agglutinin and complement fixing antibody.

My observations here are entirely in *disagreement* with those of Wagner (1917, *München. med. Wochenschr.* p. 792), who claims to have obtained positive results with the complement-fixation test using *B. proteus* as antigen in five out of six cases of Typhus so investigated.

Per contra my results showed:

(1) No increased tendency for fixation of complement by a pooled typhus serum in the presence of *B. proteus* antigen over that quantity fixed by a pooled negative serum under similar conditions.

(2) Fifty-five out of fifty-eight cases of definite Typhus Fever yielded negative complement-fixation reactions.

(3) Using an identical technique, monkeys and man after sub-cutaneous inoculations invariably yielded positive complement-fixation reactions.

[1] Craig and Fairley, *loc. cit.*

In consequence of these findings, and in contra-distinction to the generally accepted view, I hold that the only satisfactory explanation of the Weil-Felix agglutination reaction is *to regard the phenomenon as due to a secondary or heterologous agglutinin* (*Nebenagglutinin*).

It would be a matter of considerable interest to investigate the residual agglutinin in typhus sera after saturation with suspensions of *B. typhi-exanthematici* Plotz and *B. proteus* Weil and Felix. Additional data might thereby be obtained concerning the etiological significance of both micro-organisms.

Posselt and Sagasser (1903, *Wien. klin. Wochenschr.* No. 24) showed that in immunisation there is not only an increase in the amount of agglutinin for the organism injected but also of secondary agglutinins (*Nebenagglutinine*) which act on other organisms. Thus they found that while the serum of a guinea-pig immunised against *B. typhosus* contained specific agglutinin for that organism, even in a titre of 1/12,000, it also had developed secondary heterologous agglutinins for *V. cholerae* of a titre of 1 in 4500, and for *B. dysenteriae* of a titre of 1 in 4000.

Later Ballner and Sagasser showed that at times these secondary agglutinins are markedly increased and that inoculation with certain organisms like *B. tetani* and Friedländer's bacillus, while leading to the formation of but few specific agglutinins (*Hauptagglutinine*), produced numerous secondary heterologous agglutinins.

A review of the literature certainly leads to the conviction that the sera of typhus cases must be particularly rich in these secondary agglutinins.

Thus agglutinins in typhus sera have been described by the following observers for the following micro-organisms:

(1) For *B. typhi-exanthematici* by Plotz (*loc. cit.*).

(2) For *Bacillus* "U" by Wilson (*Journ. Hygiene*, 1909, IX. 316–337; *ibid.* 1910, X. 155). This organism is a variant strain of *B. coli* and was isolated from the faeces of a case of Typhus during the Belfast epidemic of 1908. Wilson attributes this agglutination to the presence of secondary heterologous agglutinin.

(3) For *Bacillus typhosus*, Wilson (*loc. cit.*) reports positive agglutination in a titre of 1 in 50 as existing in the sera of eighteen out of thirty-one cases of Typhus, and concluded that the Widal reaction was of no value in distinguishing Typhus Fever from Typhoid.

(4) Hornicki, in Manchuria, isolated an organism allied to *Bacillus* "U" of Wilson from the urine and faeces of typhus cases and reported positive agglutination reactions with the sera of typhus patients.

(5) Weil and Felix (*loc. cit.*) have described two strains of *B. proteus* (the X 2 and the X 19) which have already been referred to in detail.

Wherein lies the explanation of this almost promiscuous agglutinating action of typhus serum on a number of biologically distinct species of micro-organisms?

Surely it is impossible for *all* these organisms to be constant secondary invaders in Typhus Fever. Is it not more rational to think that the virus or specific agglutinogen of typhus, whilst stimulating homologous receptors or specific agglutinins, also has the property of stimulating other closely related receptors of secondary agglutinins, which agglutinate micro-organisms of different biological strains? Such a hypothesis would afford an explanation not only of the presence of agglutinin for *B. proteus* and the absence of specific immune body for that organism in the sera of typhus cases, but also of the wider agglutinating properties possessed by typhus sera in general.

Furthermore such a conclusion need not detract from the value of the Weil-Felix reaction as a laboratory aid to the diagnosis of Typhus Fever.

The fact that the Wassermann reaction is not a specific test for syphilis, in the strict immunological sense, has not diminished its practical application as a diagnostic test for that disease.

In similar fashion the appearance in the sera of typhus patients of the agglutinin for an organism, which has no apparent relationship to the disease, need not bias the student against the great diagnostic value of this reaction.

CONCLUSIONS.

(1) The Weil-Felix agglutination reaction has again proved, in a further series of cases, to be a very reliable laboratory aid to the diagnosis of Typhus Fever.

(2) Frequently the reaction becomes definitely established during the first week of the disease. The maximum agglutination readings are obtained during the second week of fever and during the first week of convalescence (*i.e.* 8th to 21st days).

(3) Of sixty-five cases of definite Typhus Fever sixty-three or 94 % yielded positive agglutination reactions.

(4) Of 120 non-typhus sera no case yielded positive agglutination in a dilution of 1 in 20, utilising Garrow's method of agglutination. In two cases an agglutination in a dilution of 1 in 10 was obtained. On the Garrow's agglutinometer a positive agglutination of patient's serum in a titre of 1 in 40 may be regarded as diagnostic of Typhus Fever. A positive reaction in a dilution of 1 in 20 of patient's serum may be regarded as sufficient evidence on which to isolate a case during the first week of illness.

(5) The appearance of the agglutinin in the sera of typhus cases for the *B. proteus utilised in the present investigation* is not accompanied by the formation of specific immune body. On the other hand living cultures inoculated sub-cutaneously into monkeys and man are followed, not only by the appearance of agglutinin, but also by the production of immune body as revealed by the complement-fixation test, utilising *B. proteus* as antigen.

(6) The hypothesis that *B. proteus* is a constant secondary invader accompanying the unknown virus of Typhus Fever lacks confirmation and is

incompatible with certain ascertained facts. The Weil-Felix reaction is dependent on the presence in typhus sera of a secondary non-specific agglutinin which has the property of agglutinating this *proteus*-like organism.

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