THE WILSON-WEIL-FELIX REACTION IN TYPHUS FEVER[1].

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(A REPORT TO THE MEDICAL RESEARCH COUNCIL.)

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In the first part of this communication I have shown that in Ireland it is possible to diagnose Typhus Fever by means of a serological test, thereby confirming the results obtained in Germany, Austria, Russia, Turkey, Egypt, Palestine, Mesopotamia, France, Italy, Spain, Holland, and Peru. In the second part I have traced the development of the test and have endeavoured by a review of the literature of the subject to offer an explanation of a phenomenon which opens up new ground in the field of immunity.

PART I.

Most authorities believe that the Widal test is of value in distinguishing between Typhoid Fever and Typhus Fever. From a wide experience of the test in many outbreaks of Typhus Fever I can state that in certain of these the Widal test is uniformly negative whilst in others it is in a considerable number of cases as markedly positive as if the patients were suffering from Enteric Fever. I had reached this conclusion in 1908 as the result of investigations made in conjunction with Dr E. H. Milligan. At the same time Patterson (1908) in Lanarkshire showed that the blood serum of Typhus Fever cases can agglutinate the Typhoid bacillus in high dilutions.

In 1909, I pointed out that in certain cases the sera of Typhus Fever not only agglutinated the Typhoid bacillus but also a late lactose-fermenting coliform bacillus isolated from the intestine of one of the patients. This coliform bacillus was subsequently proved to be identical with a bacillus isolated by T. Horiuchi from cases of Manchurian Typhus Fever and which were thought by this observer to be due to infection with his bacillus. I however contented myself with the statement: "The fact that the blood serum of typhus fever cases in Manchuria and in Ireland should have been independently discovered to have an agglutinative action on an intestinal organism is rather interesting, but whether the phenomenon should be taken as an instance of specific or of heterologous agglutination we must leave for the present undecided."

[1] An abstract of this paper was read before the Pathological Society of Great Britain and Ireland at its meeting in Edinburgh in July 1919.

In 1910, in a paper on the Etiology of Typhus Fever, I pointed out that:

(1) 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 three to ten times the agglutinative effect of normal serum.

(2) From the urine of two cases a bacillus resembling *B. coli communis*, but having no action on lactose, was cultivated. This bacillus formed blue colonies on the Conradi-Drigalski medium and was regarded by me as an intestinal micro-organism but whether it belonged to the *B. coli* or *B. proteus* group was not determined. This bacillus was agglutinated in dilution of 1 in 50 and 1 in 100 by the serum of the cases but not by normal serum. As to the interpretation of such facts I stated: "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."

From the above references it is obvious that as long ago as 1908 I established the presence of heterologous agglutinins for intestinal bacilli in the blood serum of Typhus Fever cases. The recent work on the Weil-Felix reaction is an amplification of my investigations, the basis of the test being the demonstration of heterologous agglutinins. The micro-organism employed by Weil and Felix is a proteus bacillus whereas of my cultures the intestinal were probably genuine varieties of the *B. coli* and the urinary varieties of *B. proteus*. It would appear from the wide use of "X 19," the strain of *B. proteus* most commonly employed by Weil and Felix and others, that this bacillus is the most suitable for the detection of these agglutinins.

In view of my initial work on the subject I would propose that the test should be known as the Wilson-Weil-Felix reaction.

Through the kindness of Dr Arkwright I was able to obtain a culture of "X 19" and to employ it in the investigation of the blood sera of 24 cases of Typhus Fever which occurred in Londonderry during December 1918 and in the early months of 1919. Dr Craig the Medical Officer of Health of Londonderry very kindly supplied me with specimens of blood.

The results of my observations may be summarized as follows:

(1) Of 23 cases examined the agglutinative titre of the sera against an emulsion of "X 19" was as follows: one in 1 in 40, four in 1 in 80, five in 1 in 160, three 1 in 320, four in 1 in 640, one in 1 in 1280, four in 1 in 2560. Most of these observations were made during the second week of the disease. In one case the reaction was negative in 1 in 20 dilution on the 5th day of the disease but a week later agglutination occurred in a dilution of 1 in 160. One case examined on the 12th and again on the 14th day was negative: the death of this patient prevented further examination. As controls sera from the following gave completely negative results in a 1 in 20 dilution: 50 cases of Influenza, 12 cases of Trench Fever and 15 cases of Syphilis.

(2) The agglutinative reaction is slow when cultures killed by heat and preserved with 0·1 per cent. formalin are employed. Results can be read after 20 hours at room temperature or after 12 hours at room temperature followed by two hours at 55° C. One of the cases showed a definite "Zone of Inhibition," agglutination being absent in dilutions of 1 in 20 and 1 in 40 but present in 1 in 80, 1 in 160, etc.

(3) The examination of 10 sera convinced me that the formation of agglutinin for this proteus-like organism in the blood of typhus cases is not accompanied by the formation of immune body, as indicated by the complement fixation reaction using as antigen a fresh saline suspension of this proteus-like organism. This is confirmatory of the work of Craig and Fairley (1918) and differs from that of some Continental observers. In carrying out the test the sera were exposed to the antigen for different periods and at different temperatures.

(4) The titre of the agglutinins in the sera of 20 cases for a coliform bacillus isolated from the urine of one of them was: one in 1 in 640, five in 1 in 320, five in 1 in 160, six in 1 in 80, three in 1 in 40. The blood serum of 50 Influenza cases and of 12 Syphilitics gave feeble agglutination in a 1 in 20 dilution at 55° C. but none in 1 in 20 at room temperature. In the case of no non-typhus serum was there agglutination with a 1 in 40 or higher dilution. The agglutinins for this coliform bacillus were best demonstrated at 50° C. This bacillus was a genuine *B. coli*. It fermented lactose and was most readily agglutinable when grown on lactose agar. Other strains of *B. coli* which were isolated from Typhus cases gave no agglutination.

(5) Absorption experiments indicated that the agglutinins for *B. proteus* "X 19" and this coliform bacillus were distinct.

(6) The agglutinins in Typhus serum for *B. proteus* "X 19" are completely destroyed by heating for half an hour at 60–65° C. whereas the specific agglutinins for the same bacillus in the serum of an inoculated rabbit are not destroyed until a temperature of 75° C. is reached.

These results confirm those of everyone who has used the test. In my controls the results were always negative in a 1 in 20 dilution. I had an opportunity through Col. H. L. Cummins kindly supplying me with Schiff's

Diagnostikum—a dead suspension of "X 19"—to test the action of Trench Fever serum on the bacillus. I had early in 1917 pointed out certain points of resemblances between the two diseases but my experiments showed me that as regards the Wilson-Weil-Felix test they were quite different. Jungmann and Kuczynski (1917) had also remarked on the close relationship of these diseases and later Kuczynski stated that he had seen "X 19" agglutinated by the serum of Volhynian Fever. I may mention that I endeavoured to develop a similar test for the diagnosis of Trench Fever, using cultures obtained from the urine of the cases, and, although I found that there was an increase in their blood of agglutinins for a bacillus which in cultural and fermentative characters resembled *B. paratyphosus* B, but which differed from the latter in forming indol, still the serum in some other conditions showed the same alteration and rendered the test unreliable.

In a case in which the clinical diagnosis of Influenza and Typhus Fever was in question I was able to decide the matter by finding agglutination of *B. influenzae* in a 1 in 160 dilution and a negative Wilson-Weil-Felix in a 1 in 20 dilution.

Other observers have used as controls the sera of patients suffering from the common tropical diseases and as a rule where Typhus infection, recent or remote, could be excluded the test was negative. The chief exception is Enteric Fever where agglutination is sometimes found in dilutions of 1 in 50 and even in 1 in 100.

From a perusal of the literature it is obvious that there has been a difference in the agglutinability of the strains of *B. proteus* "X 19" which have been employed by different observers. Indeed Diehl (1918) has already called attention to this variability. Schiff (1919) states that when "X 19" has been cultivated for some time on sugar-free media, it loses to a great extent its agglutinability and this reappears on the addition of glucose. When the bacillus is grown in a medium rich in glucose it becomes spontaneously agglutinable. Csernel (1918) claims to have rendered ordinary strains of *B. proteus* agglutinable by Typhus serum by cultivating them on lactose agar together with acid-producing faecal bacteria. I observed that strains of *B. coli* which were agglutinable by Typhus serum were rendered still more agglutinable by cultivating them on lactose agar, the lactose being fermented by them. In carrying out the test care should be taken to see that the strain of *B. proteus* "X 19" used is of a suitable degree of agglutinability. In view of the possibility of this characteristic being lost or modified by continued cultivation I have experimented with emulsions prepared from dried bacterial substance. I believe that in the dried state the bacteria preserve their agglutinable characteristics unaltered and that from such dried bacterial substance suitable emulsions can be employed for carrying out the test when a case for diagnosis crops up.

PART II.

DISCUSSION.

From a consideration of the very extensive literature dealing with the serology of Typhus Fever and from my own observations, it would now seem to be definitely established that the blood serum in this disease is capable of agglutinating many species of micro-organisms. Gram positive *Diplococci* (Wilson) and Diphtheroid bacilli, *B. typhi exanthematici* Platz (Baehr, Olitsky, Popoff), are frequently clumped but it is especially on certain strains of intestinal micro-organisms that the agglutination effect is manifested. The agglutination of certain coliform strains and of certain non-lactose fermenters probably of the *B. proteus* group occurring in the urine of patients is a phenomenon, as shown by me in 1908, characteristic of Typhus serum. On an average the sera of about 10–20 per cent. of the cases also agglutinate *B. typhosus*, *B. paratyphosus* A or B (*vide* Wilson, Patterson, Koehler, Zlocisti, Popoff, Mühlens and D. Stajanoff, Napier, Fairley, Werner and Leoneanu, Blanco and Tapia, Ficai, and Kramer). Nicolle in Tunis frequently found Typhus serum capable of agglutinating *Micrococcus melitensis*.

The recognition of heterologous agglutinins in Typhus serum for intestinal bacilli isolated from the intestine and urine of the cases was first made by the writer. Horiuchi (1908) a little earlier during the Russo-Japanese War had discovered a coliform bacillus in the urine of cases of Manchurian Fever and found that this bacillus was agglutinated by their serum. He named his bacillus "*Bacillus febris exanthematici Mandchurici*" and regarded it as the cause of the outbreak in question but whether the outbreak was to be regarded as true Typhus Fever Horiuchi left in *dubio*. The question of the agglutinins being heterologous in nature never entered his mind.

It was however the work of Weil and Felix (1916) and their followers which firmly established and led to the general recognition of the presence of heterologous agglutinins as diagnostic of Typhus Fever. The bacillus which was discovered by them and which has been found most admirable for the detection of these agglutinins in Typhus serum is a strain of *B. proteus* and has been named "X 19." The strain "X 2" which they first used and which was probably similar to that isolated from urine by me was found not to be so readily agglutinable as the latter strain. The distinction between a non-lactose-fermenting coliform bacillus and a *B. proteus* can only be made by a complete study of the micro-organisms. It is now recognised that the proteolytic power of *B. proteus* is variable—the majority liquefy coagulated serum and gelatin but some strains attack neither—others digest serum but do not liquefy gelatin (Schaeffer). Indol production is a variable character. All the *B. proteus* X strains produce indol and this indol producing capacity seems to be associated with the power to ferment maltose and saccharose (Schaeffer). Wenner and Rettger (1919) from a study of the genus *Proteus* propose that it should be divided into two species, *Proteus vulgaris* and *Proteus mirabilis*,

the former fermenting maltose and saccharose with the production of acid and gas, the latter not fermenting maltose and only very slowly, if at all, fermenting saccharose. Mannite and lactose are not fermented by *B. proteus.*

B. proteus "X 19" ferments glucose, laevulose, galactose, maltose and saccharose, liquefies gelatin and belongs to the *Proteus vulgaris* species. "In their agglutination power the members of the *Proteus* genus are heterogeneous in character so that no distinct separation into species is possible on this basis" (Wenner and Rettger). Braun and Salomon in a study of 36 *Proteus* strains of non-Typhus origin and of ten strains sent by Weil put them in three categories: (1) those possessing almost no agglutinogens common with the *Proteus* of Typhus, (2) those having agglutinogens common with all the Typhus strains, (3) the Typhus strains.

The great and only characteristic of the "X" strains and especially of "X 19" is that they are agglutinated by the serum of Typhus Fever cases. Typhus serum does not agglutinate ordinary *Proteus* bacilli, although among the latter there are strains which are agglutinated by the anti-serum of man or rabbit prepared against the "X" strains (Weil and Felix).

It is a curious fact that, although ordinary *vulgaris* strains of *B. proteus* have been isolated from Typhus cases, no agglutinins are found for these strains in the patients' sera, although the sera possess a high agglutinin content for "X" strains and all attempts in most instances to isolate the latter from patients' bodies have failed. This circumstance appears to the writer to militate against the view that in Typhus there is always specific infection with *Proteus* bacilli and that this infection is the cause of the appearance of the agglutinins in the sera. If this infection occurred it is surprising that it is for the "X" strains alone that agglutinins are found.

Another argument against the view that the agglutinins are indicative of infection is the rarity with which "X" strains have been isolated from the cases. Thus Schürer and Wolff (1919) state that Felix attempted without success on 419 occasions to cultivate *Proteus* bacilli from 250 cases of Typhus, that Zeiss in 277 blood samples found them 18 times and that Wolff out of 78 attempts was successful eight times. Schürer and Wolff (1919) in 250 blood samples from 260 typhus cases with high fever found *B. proteus* 20 times, i.e. 7·7 per cent. of the cases examined. Of these 20 only seven corresponded to "X 19" type of Weil and Felix and three to their "X 2" type.

Schürer and Wolff (*loc. cit.*) examined 450 catheter specimens of urine from 95 cases of Typhus and found *Proteus* bacilli 137 times but of these only 16 showed an agglutination with Typhus serum in higher dilutions than those of 72 strains of *Proteus* isolated from patients not suffering from Typhus Fever but from Enteric Fever, and Dysentery.

It is clear then that "X" strains are not frequently met with in Typhus Fever and they are not peculiar to Typhus but have been found in the intestine of individuals who never suffered from this disease. Georg Wolff (1919) found "X" strains three times among 116 *Proteus* strains isolated from non-

typhus patients. This is a point against the view that the "X" strains are ordinary *Proteus* strains modified by growth in the body of Typhus Fever patients.

It may be asked are these agglutinins in any way different from agglutinins developed as the result of inoculation of man or animal with "X 19"? Hamburger and Bauch (1917) believe that the agglutinins found in Typhus serum are true agglutinins but that they differ from specific agglutinins formed as a result of inoculation in that the former are completely destroyed in half an hour at a temperature of 65° C., the latter only at 75° C. This greater susceptibility to heat has been confirmed by Jacobitz (1918) and Wilson. Hamburger and Bauch found that like other agglutinins the typhus agglutinins were precipitated from the sera by ammonium sulphate and were absorbed by animal charcoal and by emulsions of the bacilli in question.

A difference between the Typhus agglutinins and specific agglutinins resulting from inoculation with "X 19" is manifested with regard to their action on heated and unheated bacilli. Dietrich (1916) showed that if emulsions of "X 19" were used which had been heated to 56° C. or which had been treated with phenol or formalin little or no agglutination occurred. Sachs confirmed this and states that the microbes become agglutinable again when the temperature is raised to 80° C. and that the bacteria now preserve their agglutinability longer than non-heated emulsions and that they are agglutinated in a higher titre although the agglutinative action is slower. Schiff found that cultures heated to 100° C. for two minutes are generally well agglutinated and that bacilli rendered inagglutinable by heating to 56° C. recover their agglutinability on being washed with normal saline solution.

Weil and Felix found "X 1" and "X 2" more agglutinogenic and much less agglutinable than "X 19" by Typhus serum. Sachs showed that the anti-sera of rabbits agglutinate all three to the same degree. When however the bacilli are heated the results differ—thus the anti-sera of "X 1" and "X 2" which agglutinate the corresponding microbes either living or heated, no longer agglutinate "X 19" when the latter has been heated: at the same time "X 1" and "X 2" become after heating inagglutinable to the serum of "X 19."

As regards the presence and production of agglutinins for "X 2" and "X 19" Felix had an interesting experience. From the cadavers of 18 Turks who had died from Typhus Fever, Felix failed to isolate any *Proteus* strains in ten, in five he found ordinary *Proteus* strains and in only three "X" strains and of these two belonged to the "X 2" and one to the "X 19" group. Now the curious fact emerged that the blood serum of the two cases which harboured "X 2" failed to agglutinate this bacillus, but agglutinated "X 19" whilst the subject from whom "X 19" was derived agglutinated both strains.

In infections with most micro-organisms the presence of an immune body can be demonstrated by means of the complement fixation test. If there is a true infection in Typhus with *Proteus* bacilli one would expect to find

complement fixed when "X 19" is used as antigen. It is unfortunate that the results obtained by different observers are conflicting and that further investigations will be required to settle the point. I failed to find any fixation and in this I am in agreement with Fairley, Otto, Dietrich and Orticoni.

Fairley's investigations were most exhaustive and would seem to be conclusive as regards the strain of "X 19" he was using as antigen. His 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) 55 out of 58 cases of definite Typhus Fever yielded negative complement-fixation reactions; (3) using an identical technique, monkeys and man, after subcutaneous inoculations, invariably yielded positive complement-fixation reactions. Fairley states his conclusions as follows: "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 heterologous agglutinin (Neben-agglutinin)."

On the other hand Friedberger (1917), von Gutfeld (1919), Werner (1918), Reichenstein (1917) and Papamarku (1917) found complement-fixation occurred with "X 19" in the majority of cases. Papamarku states that an antigen in which the bacilli were killed by phenol gave better results than where heat was employed.

As regards other effects of Typhus serum on *Proteus* "X 19" it may be mentioned that Lisbonne and Carrère (1919) found that it caused a precipitate with the filtrate of cultures of *Proteus* "X 19."

Weltmann (1917) states that when fresh Typhus serum is added to five times its volume of distilled water a turbidity occurs and that this is absent with normal serum and with Typhus serum which has been heated for ten minutes at 60° C.

Vaglio (1919) states that when *Proteus* "X 19" is grown in broth containing 1 per cent. of Typhus serum the bacilli are deposited as clumps at the bottom of the tube after three hours incubation at 37° C.

As to the explanation of the presence of agglutinins for *Proteus* "X 19" in Typhus serum hardly anyone now considers that this micro-organism is the cause of Typhus. Apart from other arguments the negative results of the human inoculation experiments of Kuczynski and Fairley are conclusive.

The great majority of those who have studied the subject incline to the view that the agglutinins are due to a constant secondary infection with *Proteus* "X 19." This may be true but we have seen in the preceding discussion some points against this hypothesis. I have found (and this is a matter that has recently been confirmed by Schaeffer) that agglutination of certain strains of *B. coli* is almost as constant and as specific an effect of Typhus serum as the agglutination of *Proteus* "X 19." I have also found that absorption by "X 19" leaves agglutinins for *B. typhosus* and for agglutinable *B. coli*

strains unaffected. Absorption with *B. typhosus* and with the *B. coli* strains that I have studied also leaves the agglutinins for *Proteus* "X 19" undiminished in amount. If these agglutinins for intestinal micro-organisms are due to a subinfection (and it is difficult to understand how otherwise they could arise) then the infecting micro-organism must be a species that is constantly present in the bodies of the patients. It would appear that "X" strains are rarely met with and that it is unusual to get agglutinins for the *Proteus vulgaris* strains which are frequently found in the human intestine. It appears to the writer probable that the Typhus Fever virus renders the intestine more permeable to intestinal micro-organisms, and that certain strains of *B. coli* or allied organisms infect the patient and lead to the formation of specific agglutinins for themselves and of paragglutinins for other intestinal micro-organisms, e.g. *Proteus* "X 19," "X 2," coliform bacilli, *B. coli*, *B. typhosus*, *B. paratyphosus* A and B, *Micrococcus melitensis*, etc. It is of some bearing in this connection that occasionally in Enteric Fever there is agglutination of *Proteus* "X 19" in 1 in 100 dilution. When this micro-organism is discovered and isolated it will probably be found that it produces a specific agglutinin and immune body for itself and paragglutinins for a great variety of other bacteria and that absorption of the serum with this culture will remove not only the specific agglutinins but also the paragglutinins.

It has been proved by Möllers and Wolff (1919) and by Ribeyro (1919) that inoculation of guinea-pigs with Typhus virus is not followed by the development of agglutinins for "X 19." Doerr and Pick (1919) state that when rabbits were inoculated with Typhus virus, they presented no signs of infection but that the Typhus virus became located in their brains and their blood serum agglutinated *Proteus* "X 19" and *B. typhosus* in dilutions up to 1 in 60 and 1 in 40 respectively.

Some light is thrown on the Wilson-Weil-Felix reaction in Typhus Fever by the study of the formation of group and heterologous agglutinins in other diseases. Kligler (1918) reported the cross-agglutination of *B. coli communis* and *B. dysenteriae* Shiga. The end point of the titre of the serum of inoculated rabbits was in each case 1 in 4000. The property of reciprocal agglutination was limited to the two strains described. Other cultures of *B. coli* and *B. dysenteriae* Shiga did not exhibit it. Absorption experiments made with each culture upon each kind of immune serum indicated that two distinct agglutinins were yielded in about equal amount in the process of immunization of rabbits with the respective cultures. The two agglutinins were specific ones, each for its own culture, and accessory (paragglutinin) each for the other culture. The absorption of the accessory agglutinin left the specific agglutinin quantitatively unaffected, but absorption of the specific agglutinin by its own culture completely removed the paragglutinin.

Park and Williams (1910) observed that the serum of a horse immunized with *B. dysenteriae* Flexner agglutinated *B. coli* in the same end dilutions (1 : 10,000) as the dysenteric bacillus. Conversely a goat immunized with

B. coli yielded a serum of a titre of 1 : 5000 for the *B. coli* and 1 : 3000 for *B. dysenteriae* Flexner.

Of great interest is a paper by Frost (1910) on *Pseudomonas protea* which he isolated from water and found to be agglutinated by the serum of Typhoid Fever patients. This organism which is closely allied to *Proteus* was agglutinated by the serum of Typhoid Fever cases in a larger percentage of cases than was *B. typhosus*, the difference being especially marked in the early stages of the disease. The serum of cases of Typhoid Fever often agglutinated *Ps. protea* in higher dilutions than it agglutinated *B. typhosus*. The serum of four cases of Paratyphoid Fever also agglutinated the micro-organism. Specific Typhoid immune-serum from animals in the early states of immunization, agglutinated *B. typhosus* (1 in 2000) but later as the agglutinating strength for *B. typhosus* increased, the titre for *Ps. protea* failed to increase proportionately. The results of absorption experiments indicated that the agglutination of *Ps. protea* by specific Typhoid agglutinating serum is effected by combination with a portion of the specific Typhoid agglutinin; that it was therefore a "group" agglutinin. Animals injected with culture of *Ps. protea* developed agglutinins for this organism but none for *B. typhosus* or other organisms of the Colon-typhoid group.

These observations of Frost would tend to support the view put forward in this paper that the agglutinins for *Proteus* "X 19"—an organism closely allied to the *Ps. protea*—may prove to be group agglutinins of some organism of the Colon group which is a constant inhabitant of man's intestine. It is noteworthy in Frost's experiments that inoculation with his *Ps. protea* produced no group agglutinins for *B. typhosus* but that inoculation with the latter produced group agglutinins for the *Ps. protea*.

In an endeavour to fix in its true setting the Wilson-Weil-Felix reaction in the field of Immunity, I believe help may be derived from the contents of our three papers in the *Journal of Hygiene* (Symmers and Wilson, 1908; Wilson, 1909 and 1910). The following quotations from the second paper appear to me to bear on the problem under discussion: "In round numbers 90 % of the cases of Cerebro-spinal fever agglutinated the *B. aquatilis alkaligenes* in a dilution of 1 in 50. In most cases agglutination still occurred with a 1 in 100 and higher dilutions. In 16 cases the blood serum gave marked 'clumping' within an hour to dilutions of 1 in 1000 and further we found that one of the 16 agglutinated in 1 in 1400, two in 1 in 1500, four in 1 in 1600 and four in 1 in 2000 dilutions respectively. We have shown that it is possible to remove the agglutinins from the serum by saturation with the *B. aquatilis alkaligenes*, whilst saturation with the *Meningococcus*, *B. typhosus*, *B. coli communis*, or *B. faecalis alkaligenes* (Král) leaves them intact."

"Of 31 specimens of the serum of normal adults examined three, i.e. 9·6 per cent. gave a positive reaction in a 1 in 50 and all a negative reaction in a 1 in 100 dilution of the serum." The *B. aquatilis alkaligenes* was a non-pathogenic micro-organism which had been isolated from tap water. This curious instance

of heterologous agglutination by means of which we were enabled to diagnose cases of Cerebro-spinal Fever is worthy of note.

At that time (1908) I succeeded in only one case in isolating a similar bacillus from the blood of the patients and my examinations of the Cerebro-spinal fluid, urine and blood were negative as regards the *B. aquatilis alkaligenes*. However, in 1915, in an outbreak of Cerebro-spinal Fever, I frequently found associated with the *Meningococcus* in the spinal fluid a bacillus which was evidently of intestinal origin and which fermented no sugar but usually liquefied gelatin. This bacillus although allied to the *B. aquatilis alkaligenes* was not agglutinated by the serum of the cases in nearly so high a titre as the latter. It in fact bore much the relationship to the *B. aquatilis alkaligenes* that *Proteus* "X 1," "X 2" and ordinary *Proteus vulgaris* strains bear to "X 19."

"Paltauf (1904) says the results of Posselt and Šagasser (1903) as well as those of Hetsch and Lentz go to show that in the immune serum of animals as well as in that of sick men and women, heterologous agglutinins exist which have no binding groups for the infecting bacteria but are as specific as regards absorption as those developed in a mixed infection. They must therefore be distinguished from partial agglutinins or 'mitagglutinins.' They can be designated as 'heterologous nebenagglutinins' or more briefly as 'neben-agglutinins.' For their formation the views held regarding partial agglutinins do not apply." "The explanation of the fact that the blood serum of Europeans contains specifically absorbable agglutinins for the *V. cholerae* and *B. pestis* may be that these agglutinins are partial agglutinins or 'nebenagglutinins' caused by the action of unknown sapropbytic intestinal organisms."

I would suggest that a similar explanation may account for the agglutinins present in Typhus serum for *Proteus* "X 19," etc. With regard to the agglutinins in Cerebro-spinal Fever for *B. aquatilis alkaligenes* and *B. typhosus* I concluded that "these secondary agglutinins do not indicate a mixed infection but are of the nature of heterologous 'nebenagglutinine.'" I proved that they were not partial agglutinins due to infection with the *Meningococcus* but I now think it not improbable that they were of the nature of partial agglutinins for some unknown intestinal micro-organism. I indeed stated that "the explanation of the production of heterologous agglutinins may be that infection with certain germs leads to an alteration of the bacterial flora of the intestine. As the result of this secondary auto-infection, along with the agglutinins for the primary infecting organism, agglutinins are formed for the intestinal micro-organisms also." Experiments conducted by Elser and Huntoon tend to confirm the statements made above and to offer a similar explanation as is made clear by the following extract from their paper: "In rabbits we have shown that injections of the Meningococcus render the mucosa of the intestinal tract more permeable for the Typhoid bacillus (or its products) administered with the food. Animals thus treated developed agglutinins for the organisms administered by mouth, while no augmentation of the agglutinins was

observed in the controls which were fed with Typhoid bacilli but received no injections. These experiments suggest an explanation for the frequent occurrence of mixed infections in animals receiving experimental inoculations of the meningococcus, and also offer an explanation for the appearance of heterologous agglutinins in the sera of such animals and in the sera of individuals suffering from epidemic cerebro-spinal meningitis."

It is pertinent to note that with regard to the heterologous agglutinins occurring in Cerebro-spinal Fever I had shown that (1) they are bound up with the globulin component of the serum, the greater part belonging to the "pseudo-globulin" fraction; (2) by heating the serum to 60° C. their destruction is complete. Similar results have been obtained in the case of the agglutinins for "X 19" in Typhus serum.

Before concluding I should mention that Epstein regards the different reactions given by Typhus serum as due to changes in the physico-chemical state of the serum. He has shown that the albumin-globulin ratio in Typhus varies from 0·46 to 0·8 whereas in other pathological states it exceeds unity. However it is difficult to conceive how apart from subinfection with intestinal bacilli this change should lead to the production of absorbable agglutinins for these micro-organisms. No doubt the action of agglutinins is best explained in terms of physical chemistry but an explanation of their production apart from infection with some allied micro-organism is difficult. The agglutinins for *B. aquatilis alkaligenes* I showed were heterologous in the sense that they were not partial agglutinins, group agglutinins, or agglutinins due to infection by the *Meningococcus* acting alone. The agglutinins in Typhus serum are also heterologous as regards their production by the virus of Typhus but in both instances I would suggest that they are due to subinfection from the intestine. The infecting micro-organisms however have, so far, not been definitely determined and are unlikely to be *B. aquatilis alkaligenes* or *Proteus* "X 19" but some constant inhabitants of the intestine which under the stimulus of the disease produce specific agglutinins for themselves and group agglutinins for numerous other micro-organisms especially for *Proteus* "X 19," *Proteus* "X 2," and various members of the Colon-typhoid group.

Summary.

1. The Wilson-Weil-Felix agglutination reaction with cultures of *Proteus* "X 19" was Positive in dilutions of the serum ranging from 1 in 40 to 1 in 2560 in 22 out of 23 cases of Typhus Fever occurring in Ireland. In one case the reaction was still negative at the time of the patient's death on the 14th day of the disease. As controls, sera from the following gave completely negative results in a 1 in 20 dilution: 50 cases of Influenza, 12 cases of Trench Fever and 15 cases of Syphilis.

2. The agglutinins in Typhus serum for *B. proteus* "X 19" are completely destroyed by heating at 65° C. for half an hour.

3. Ten sera were tested for presence of immune body by means of the Complement Fixation test. The results were negative.

4. A coliform bacillus isolated from the urine of one case was agglutinated by the sera of twenty cases—the only ones examined—in dilutions varying from 1 in 40 to 1 in 640.

Sixty-two controls gave negative results with a 1 in 40 or higher dilution of their sera.

5. Absorption experiments indicated that the agglutinins for *B. proteus* "X 19" and this coliform bacillus were distinct.

6. The literature dealing with the heterologous agglutinins met with in the serum of Typhus Fever has been consulted and the various hypotheses that have been put forward to account for their presence have been examined. It is suggested that under the stimulus of infection with the Typhus Fever virus, some bacterium which is a normal inhabitant of the human intestine produces agglutinins for itself and group agglutinins for numerous other micro-organisms especially for *Proteus* "X 19," *Proteus* "X 2," and various members of the Colon-typhoid group.

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