A FURTHER CONTRIBUTION TO THE SEROLOGY OF TYPHUS FEVER.

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In this paper I am bringing forward further evidence in support of my view that heterologous agglutinins for certain agglutinable strains of bacteria of various species occur in the blood serum of typhus fever cases, and that it is improbable that "X 19" or other member of the *Bacillus proteus* group is specifically associated in any way with the etiology of the disease. A quotation from a former paper (1920) will make my position clear. "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 the present paper I shall show that a bacillus of the colon-paratyphoid group in the majority of the cases in which I have had an opportunity of putting it to the test during the past 5 years has been as sensitive an indicator as "X 19," and I write in the hope that others may use the bacillus and ascertain whether on a more extended trial it fulfils the purpose usually so admirably served by "X 19." As this bacillus is very closely related to the bacillus "U" originally employed by me I have named it *B. agglutinabilis* "U_2." It was isolated from the stools of a typhus fever patient (E. T.) on 27. XI. 21, and throughout the five years up to the time of writing it has been found to be as readily agglutinated by typhus fever serum as at the start. The persistence of the agglutinability over a long period is a very important consideration, and it is this characteristic that has rendered "X 19" so valuable. Below is expressed in tabular form the agglutination of this bacillus and of "X 19" by the serum of 2 cases of typhus fever. These cases were the only ones which occurred in Belfast at the time and the patients were brother and sister. In the test emulsions of living bacilli from agar slopes were employed, and the readings were taken after 4 hours at 40° C. The dates refer to the time at which the blood was obtained from the cases.

It is observed that the agglutinins for bacillus "U_2" and "X 19" increase in the course of the disease, reaching a maximum about the time of the crisis

Table I.

Bacillus "U_2"

Serum E.T.	Dilutions of serum: 20	40	80	160	320	640	800	1600	3200	6400	8000	12,800	30,000
26. XI. 21	*	.	.	.	.	.	.	.	.	.	.	.	.
1. XII. 21	***	***	***	***	***	***	***	***	*	.	.	.	.
5. XII. 21	***	***	***	***	***	***	***	***	***	***	**	.	.
16. XII. 21	***	***	***	***	***	***	***	***	.	.	.	.	.
A.T.													
26. XI. 21	***	***	***	***	.	.	.	.	.	.	.	.	.
1. XII. 21	***	***	***	***	***	***	***	***	***	***	***	***	.
5. XII. 21	***	***	***	***	***	***	***	***	***	***	**	*	.
16. XII. 21	***	***	***	***	***	***	***	**	.	.	.	.	.
22. XII. 21	***	***	***	***	***	***	**	.	.	.	.	.	.

Bacillus "X 19"

Serum E.T.	Dilutions of serum: 20	40	80	160	320	640	1280
26. XI. 21	.	.	.	.	.	.	.
1. XII. 21	***	***	***	*	.	.	.
5. XII. 21	***	***	***	***	***	*	.
16. XII. 21	***	***	**	*	.	.	.
A.T.							
26. XI. 21	***	**	.	.	.	.	.
1. XII. 21	***	***	***	**	*	.	.
5. XII. 21	***	***	***	***	***	****	.
16. XII. 21	***	***	***	**	*	.	.
22. XII. 21	***	**	.	.	.	.	.

(5. XII. 21). For the sake of uniformity the readings have been taken after 4 hours at 40° C. but higher readings were obtained in some cases after the tubes had remained all night at room temperature, *e.g.* serum of A. T. 5. XII. 21 showed a trace of agglutination of "U_2" under these conditions in a dilution of 1 in 25,600.

In order to guard against any variation in the agglutinability of the emulsions used, samples of serum of different dates were tested at the same time with the same living emulsion.

As controls 15 sera which had been sent in for a Wassermann test were employed. Of these 6 showed no agglutination in 1 in 10 or higher dilution, 5 were agglutinated in 1 in 10 but not in 1 in 20, 3 were agglutinated in 1 in 20 and slightly in 1 in 40, and one showed fair agglutination in 1 in 40 and a trace in 1 in 80. At this time no further cases of typhus fever occurred in Ulster, but I had available serum from cases of typhus fever of an outbreak one year earlier. These specimens of sera were stale and their agglutination of "X 19" at this late date was feeble but 9 out of 11 examined agglutinated bacillus "U_2" in 1 in 100 dilution. I may add that the serum of case E. T. 5. XII. 21 failed to agglutinate *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, *B. aertrycke*, *B. enteritidis* (Gaertner), and a laboratory strain of *B. coli* in 1 in 50 dilution.

The bacillus "U_2" was kept alive on agar slopes, being transplanted about once every 2 months and on 3. IV. 23 I had an opportunity of testing with it

serum from cases in a small outbreak which occurred at this time in Londonderry. The results were positive and established two important points: (1) that the agglutinability of the bacillus had remained unchanged from 27. XI. 21, and (2) that the agglutinins were present in cases occurring 90 miles away from the seat of the original outbreak.

Table II.

Patient's name	Date	Day of disease	*Bacillus* "U_2" 20	40	80	160	320	640	1280	2560	5120	10,240	20,480
Mrs McC.	3. IV. 23	14th	***	***	***	***	***	***	***	***	***	*	.
	8. IV. 23	19th	***	***	***	***	***	***	***	**	.	.	.
	29. IV. 23	40th	***	***	***	**	*	*	.	.	.	.	.
W. F.	3. IV. 23 died	10th	***	***	***	***	***	***	*	.	.	.	.
J. McC.	24. IV. 23		***	***	***	***	.	.	.	.	.	.	.
	29. IV. 23	(crisis)	***	***	***	***	***	***	*	.	.	.	.
	19. V. 23		***	***	***	***	**	.	.	.	.	.	.

		Bacillus "X 19" 20	40	80	160	320	640	1280
Mrs McC.	3. IV. 23	***	***	***	**	**	.	.
	8. IV. 23	***	***	***	***	*	.	.
	29. IV. 23	***	***	*	*	.	.	.
W.F.	3. IV. 23	***	***	***	**	.	.	.
J. McC.	24. IV. 23	***	*	.	.	.	.	.
	29. IV. 23	***	***	***	***	***	.	.
	19. V. 23	***	**	.	.	.	.	.

There were only 4 cases in the outbreak: from the fourth case, being a baby, it was not possible to obtain blood. One of the contacts developed a temperature but the serum showed absence of agglutinins for "X 19" and for "U_2" and clinically the condition was not that of typhus fever.

As controls 16 sera were employed; 13 were sent in for Wassermann test, and of these only 1 showed slight agglutination in 1 in 20 dilution and none in 1 in 40. The sera of 2 cases of scarlet fever gave feeble agglutination in 1 in 20 but not in 1 in 40, and the serum of a tuberculosis case was negative in 1 in 20 dilution.

A specimen of a feebly agglutinating typhus fever serum was kindly sent me by Dr Arkwright from a case which occurred in London in April 1922. At the same time I tested an old serum which I had obtained from a case on 23. IV. 19 with the following results:

Table III.

	Bacillus "U_2" 40	100	150	200	400	600	800	1000	1200	*Bacillus* "X 19" 40	80	160	320
London serum IV. 22	***	***	*	.	.	.	.	.	.	***	**	*	.
Belfast serum B. 23. IV. 19	***	***	***	***	***	***	**	*	.	***	*	.	.

These results proved that in normal blood *B. agglutinabilis* "U_2" was agglutinated in very low dilutions by non-typhus serum, but that in typhus

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blood the agglutinins enormously increased until the crisis, after which they diminished. Further investigations were required to ascertain if this increase constantly occurred but sufficient had been done at this period to show that the serum of typhus fever cases contained not only agglutinins for *B. proteus* "X 19" but also for a coliform bacillus and that this coliform bacillus had preserved its agglutinability for over $1\frac{1}{2}$ years.

Saturation experiments showed that the agglutinins for "U_2" and for "X 19" were separate and distinct.

Characters of Bacillus "U_2."

The micro-organism was isolated by planting out faeces from a typhus fever patient on bile salt lactose neutral red agar containing in each 100 c.c. 0·4 c.c. of a 1 per cent. watery solution of brilliant green—a medium which I had found (1918) useful for the isolation of non-lactose fermenting bacilli from stools.

The bacillus formed clear colonies on MacConkey's medium and on agar and gelatin it had the cultural characters of the colon-paratyphoid group, and not the spreading growth so often found in cultures of *B. proteus*. Gelatin and coagulated serum were not liquefied. In bouillon there was produced a uniform turbidity and the indol test was negative. In young cultures the bacilli were actively motile. Glucose, laevulose and mannite were fermented with the production of acid and gas, but no action was shown on lactose, dulcite, adonite and salicin.

In Wilson's glucose sulphite iron agar medium no darkening occurred. Maltose and saccharose were very slowly fermented. On bile salt saccharose neutral red plates the colonies after a time developed papillae, and it was possible to select organisms that rapidly or very slowly fermented saccharose. The agglutinability of the slow and the rapid fermenting saccharose colonies both in relationship to typhus fever agglutinins and to specific agglutinins was found to be the same. In saccharose both the rapidly fermenting colonies formed acid in 24 hours, whereas with the slow fermenters a week elapsed before the medium became acid and a pellicle was formed on the surface of the bouillon.

The agglutination of the two varieties of the bacillus by serum obtained from rabbits inoculated with cultures is given in the table. The rapid fermenter is labelled S + and the slow fermenter S −.

Table IV.

Serum	Dilutions of serum						
	50	100	200	400	800	1600	3200
"U_2" S on S +	***	***	***	***	***	**	.
S −	***	***	***	***	***	**	.
"U_2" S − on S −	***	***	***	**	.	.	.
S +	***	***	***	**	.	.	.

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Towards the end of August 1923 through the kind services of Prof. L. Rajchman I had an opportunity of testing the sera of 8 Warsaw cases of typhus fever.

The blood of the first six cases was taken on the 9th day of the disease, and that of the seventh and eighth on the 13th and 15th days. Living cultures and also bacilli that had been preserved in alcohol were employed with identical results.

Table V.

Bacillus agglutinabilis "U_2"

	40	80	160	320	640	1280	
Case 1	***	*	.	.	.	.	4 hrs at 40° C.
	***	***	*	.	.	.	All night at room temp.
„ 2	***	***	***	*	.	.	
	***	***	***	***	.	.	
„ 3	**	.	.	.	.	.	
	***	*	.	.	.	.	
„ 4	***	***	***	***	**	.	
	***	***	***	***	***	**	
„ 5	***	***	***	***	*	.	
	***	***	***	***	***	***	
„ 6	**	.	.	.	.	.	
	***	**	.	.	.	.	
„ 7	***	**	**	.	.	.	
	***	***	***	*	.	.	
„ 8	***	***	*	.	.	.	
	***	***	***	*	.	.	

"X 19"

	40	80	160	320	640	1280
Case 1	*	.	.	.	.	.
	***	***	*	.	.	.
„ 2	.	.	.	.	.	.
	.	.	.	.	.	.
„ 3	***	***	**	*	.	.
	***	***	***	***	.	.
„ 4	*	.	.	.	.	.
	***	***	.	.	.	.
„ 5	*	*	.	.	.	.
	***	*	.	.	.	.
„ 6	*	.	.	.	.	.
	***	*	.	.	.	.
„ 7	*	.	.	.	.	.
	**	***	**	.	.	.
„ 8	**	*	.	.	.	.
	***	***	*	.	.	.

In October 1923, I received from Mexico two typhus sera through the kindness of Dr Holt-Harris and Dr Mooser; one of these was contaminated and showed no agglutinins either for "X 19" or for "U_2," the other agglutinated "X 19" in a dilution of 1 in 1280 but "U_2" only in 1 in 80. A third sample received from Dr Mooser 14. IV. 24 agglutinated "X 19" in 1 in 640 and "U_2" in 1 in 40 dilution.

About the same time Dr Altounyan was good enough to send me the serum of a case from Aleppo, Syria. This agglutinated both "X 19" and "U_2" in a dilution of 1 in 320.

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A typhus serum sent to me by Dr Walsh, Galway, showed a titre of 1 in 1280 for "X 19" and of 1 in 80 for "U_2."

From these few specimens it would seem that Mexico cases produce more agglutinins for "X 19" than for "U_2" and that cases in Poland and Syria exhibit about the same agglutinins for "X 19" and for "U_2."

I had no opportunity of carrying out further tests until October 1926, when a small outbreak of typhus fever occurred in Newry, a town about 40 miles south of Belfast.

The diagnosis of typhus in this outbreak was made accidentally. A specimen of serum had been sent to me for a Widal test from a patient on the 14th day of his disease and as the result was negative to both *B. typhosus* and to *para.* B, I tried its effect on *B. agglutinabilis* "U_2" and found prompt agglutination. Further tests with "X 19" confirmed the diagnosis and investigation showed that some cases which had been regarded as suffering from influenza were in reality infected with typhus fever.

The titre of the sera tested from Newry was as follows:

Table VI.

Patient's name	Day from onset	*B. agglut.* "U_2" 40	80	160	320	640	1280	2560	5120	10,240
T. McC.	14th	***	***	***	***	***	***	***	**	.
Mrs McC.	27th	***	**	.	.	.	.	.	.	.
P. McC	21st	***	***	***	***	***	**	.	.	.
H. McC.	15th	***	***	***	***	***	***	***	***	*
O. McC.	14th	***	***	***	***	***	.	.	.	.
J. C.	10th	***	*	.	.	.	.	.	.	.
J. C.	30th	***	***	***	.	.	.	.	.	.

Patient's name	Day from onset	"X 19" 40	80	160	320	640	1280	2560
T. McC.	14th	***	***	***	***	***	.	.
Mrs McC.	27th	***	**	.	.	.	.	.
P. McC.	21st	***	**	.	.	.	.	.
H. McC.	15th	***	***	***	*	.	.	.
O. McC.	14th	***	***	***	***	***	**	.
J. C.	10th	*	.	.	.	.	.	.
J. C.	30th	***	***	***	***	***	***	.

The readings in Table VI were recorded after 4 hours at 40° C.; after standing all night agglutination was present in somewhat higher dilutions.

As controls the 12 sera (7 sent in for Wassermann test and 5 for Widal test) were all negative in 1 in 40 and the majority negative in 1 in 10 or 1 in 20.

The same sera tested with 4 cultures of "X 19" which the Director of the National Collection of cultures kindly supplied gave the following titre under the same conditions.

There is evidently a variation in the agglutinability of the various strains. The strain Jerusalem in its present state is useless for the detection of the typhus agglutinins.

In the tests as already stated emulsions of living bacilli from agar cultures

Table VII.

Patient's name	"X 19" cultures designated	40	80	160	320	640
T. McC.	0	***	*	.	.	.
	Jerusalem	.	.	.	.	.
	No. 67	***	**	.	.	.
	Warsaw H.	*	.	.	.	.
Mrs McC.	0	***	*	.	.	.
	Jerusalem	.	.	.	.	.
	No. 67	*	*	.	.	.
	Warsaw H.	.	.	.	.	.
P. McC.	0	***	.	.	.	.
	Jerusalem	.	.	.	.	.
	No. 67	.	.	.	.	.
	Warsaw H.	.	.	.	.	.
H. McC.	0	***	***	*	.	
	Jerusalem	.	.	.	.	
	No. 67	***	***	***	*	
	Warsaw H.	***	**	*	.	
O. McC.	0	***	***	***	***	.
	Jerusalem	.	.	.	.	.
	No. 67	***	***	***	***	.
	Warsaw H.	***	***	***	***	.
J. C. 10th day	0	*	***	*	.	.
	Jerusalem	.	.	.	.	.
	No. 67	***	***	***	.	.
	Warsaw H.	.	.	.	.	.

were employed, but in nearly all cases I also made use of emulsions of bacilli which had been preserved in alcohol and found the results identical, the onset of agglutination being in most cases equally rapid and the titre the same.

In April 1923 I observed that when the growths of "X 19" and *B. agglutinabilis* "U_2" were washed off from agar plates with absolute alcohol the bacilli were clumped and settled to the bottom of the test tube. A little of the deposit taken up in a pipette and shaken up with saline gave a beautiful suspension free from clumps and readily agglutinable with typhus serum. Later I discovered that Bien and Sonntag (1917) had already employed a somewhat similar method. Their procedure was to make a thick emulsion in control saline of the growth and to leave it standing for 24 hours. They then poured off the supernatant fluid and added an equal volume of alcohol to the deposit. At the end of another 24 hours sufficient of the supernatant fluid was poured off to render the diagnostikum so thick that when 0·1 c.c. of it was added to $\frac{1}{2}$ cm. of serum a definite turbidity was produced.

In my method the bacilli are preserved in alcohol in tightly stoppered bottles. There is a deposit at the bottom of the bottle with clear spirit above. A little of the deposit, *e.g.* 0·1 c.c., taken up with a pipette and added to about 10 c.c. of saline gives a suitable emulsion. The bacilli in the bottle have not been treated with saline or with carbolic but only with absolute alcohol or spirit.

I made up bottles of "X 19" and of *B. agglutinabilis* "U_2" in May 1923 and tests carried out periodically up to the time of writing have shown that the alcoholised bacilli have remained as agglutinable as they were at the time of their preparation.

As example the serum of A. T. of 5. XII. 21 which had been preserved by the addition of an equal volume of pure glycerine was tested against *B. agglutinabilis* "U_2" on 29. IV. 23.

Table VIII.

Organism	40	80	160	320	640	1280	2560	
B. agglutinabilis living	***	***	***	***	***	***	*	2 hrs at 42° C.
	***	***	***	***	***	***	**	6 hrs at 42° C.
„ (alcoholised)	***	***	***	***	***	***	*	2 hrs at 42° C.
	***	***	***	***	***	***	**	6 hrs at 42° C.

One may conclude that the alcoholised bacilli are agglutinable and that the agglutinins are well preserved in the glycerinated serum.

The latter point is also illustrated by the following experiments. The serum of A. T. to which an equal volume of glycerine was added on 5. XII. 21 was tested on 28. X. 26 against various strains of "X 19" and against *B. agglutinabilis* "U_2" with the following results:

Table IX.

Organism	80	160	320	640	1280	2560	5120	10240	
0	***	***	**	.	.	.	.	.	4 hrs at 42° C.
Jerusalem	**	.	.	.	.	.	.	.	
67	***	***	**	**	.	.	.	.	
Warsaw	***	***	***	.	.	.	.	.	
"X 19" (our lab. strain)	***	***	***	.	.	.	.	.	
B. agglutinabilis fresh saline emulsion	***	***	***	***	***	***	**	.	
B. agglutinabilis (alcoholised)	***	***	***	***	***	*	.	.	

The fresh serum of P. McC. was on 28. X. 21 tested against a fresh living culture of "X 19" and against alcoholised bacilli, the latter having been left in alcohol since 4. V. 23. In both cases agglutination occurred in 1 in 640 dils.

Fletcher and Lesslar (1925 and 1926) find that the sera in cases of tropical typhus occurring in the Federated Malay States are of two kinds:

(1) One agglutinating ordinary "X 19" strains which belong to the indologenes group.

(2) The second with little or no effect on these strains but agglutinating an anindologenes strain designated "Kingsbury." This strain as far as its history could be ascertained, had been obtained from the National Collection of type cultures in 1921 and had apparently been isolated from a typhus fever case.

Dr Fletcher very kindly sent me a culture of the Kingsbury strain and I was able to compare it with other "X 19" strains. I confirmed his statement that unlike the other "X 19" strains it did not ferment saccharose or maltose and it did not form indol.

Rochaix and Sarda (1926) consider that in their ability to ferment aesculin and salicin and in their failure to ferment laevulose, "X 19" strains differ

from those of ordinary Proteus. I find that all the strains of "X 19" with the exception of Kingsbury ferment salicin and that other Proteus strains were inactive. As regards laevulose—many other strains of Proteus besides "X 19" have no apparent action on this carbohydrate.

The action of a few specimens of typhus serum which had been preserved by the addition of equal parts of glycerine was tested against "Kingsbury" with results shown in Table X.

Table X.

		Organism	40	80	160	320	640	1280	2560	5120
Belfast	A.T.	"X 19"	***	***	***	***	***	***	.	.
5. XII. 21		Kingsbury	***	***	.	.	.	.	.	.
	E.T.	"X 19"	***	***	***	.	.	.	.	.
		Kingsbury	.	.	.	.	.	.	.	.
Mexico		"X 19"	***	***	***	***	***	*	.	.
IX. 23		Kingsbury	.	.	.	.	.	.	.	.
Galway		"X 19"	***	***	***	**	.	.	.	.
IV. 24		Kingsbury	.	.	.	.	.	.	.	.
Newry	T.C.	"X 19"	***	***	***	***	***	***	.	.
26. XI. 26		Kingsbury	***	.	.	.	.	.	.	.

Of the 5 sera, 3 gave no agglutination of the Kingsbury strain in 1 in 40, one was positive in one in 40 and 1 in 1 in 80.

It was found by the following saturation experiments that the Kingsbury strain though agglutinated in only a low titre was able to remove the agglutinins not only for itself but also for "X 19" from typhus sera.

Saturation experiment.

The glycerinated typhus sera A. T. and T. C. were diluted 1 in 10 and to the dilutions several platinum loopsful of growth from agar cultures were added. The watch glasses were gently rocked and then left all night at room temperature.

Table XI.

	Organism	40	80	160	320
T.C. serum	"X 19"	.	.	.	.
Saturated with "X 19"	Kingsbury	.	.	.	.
T.C. serum	Kingsbury	.	.	.	.
Saturated with Kingsbury	"X 19"	**	**	.	.
A.T. serum	"X 19"	.	.	.	.
Saturated with "X 19"	Kingsbury	.	.	.	.
A.T. serum	Kingsbury	.	.	.	.
Saturated with Kingsbury	"X 19"	.	.	.	.

Fletcher and Lesslar had found that an immune rabbit serum prepared with the anindologenic strain "Kingsbury" agglutinated the other "X 19" strains of the National Collection to almost full titre and that absorption with any of these strains removed all the agglutinins from this serum. They found however that sera homologous for the other strains agglutinated the Kingsbury strain only to 12 to 25 per cent. of the full titre and that absorption with Kingsbury did not remove these agglutinins.

Our own experiments would seem to indicate that as regards our typhus sera the agglutinins for Kingsbury and the "X 19" strains were identical

but that Kingsbury although able to absorb these agglutinins was not so readily agglutinable and so was not so delicate an indicator of their presence.

Discussion.

In spite of the numerous contributions to the question, the explanation of the presence of agglutinins in typhus fever serum remains as obscure as it was in 1909 when I designated those found for certain intestinal bacilli as "heterologous."

Rocha-Lima (1919) in a very comprehensive review of the aetiology of typhus fever discussed the theories that had been advanced up to that date to explain the phenomenon.

Weil and Felix regarded the "X 19" Proteus strain as specific for the test, although they were unable to explain why the agglutinins for "X 2" and for "X 19" both in typhus serum and in immunised animals were entirely different as shown by the absorption test (Braun and Salomon, 1919). Weil (1922) endeavoured to prove that the "X_2" was a variant of the "X 19" strain. An unbiassed study of the literature will lead to the conclusion that agglutinins for certain strains of many groups of bacteria occur in the serum of the typhus fever patient and that although "X 19" is usually the best micro-organism for their demonstration still other germs in certain outbreaks are almost as sensitive, *e.g.* bacillus "U," and *B. agglutinabilis* "U_2" (Wilson), *B. pyocyaneus* (Kreuscher, 1918), Neukirsch and Kreuscher (1919), Sampietro (1920), Wilson (1922), *Coccobacillus byzantinus* (Béguet 1921).

This coccobacillus Béguet found much more sensitive for the detection of typhus fever agglutinins than three strains of "X 19." He described it as a short non-motile coccobacillus with growth on agar of the colon type. It did not liquefy gelatin or serum and formed no indol. It formed no acid or gas in lactose, laevulose and mannite and formed acid and gas in glucose, saccharose, maltose and galactose media.

Perhaps it might be regarded as an "0" strain of "X 19." Then we have the important observation of Fletcher and Lesslar (1926), that in about 50 per cent. of the cases of tropical typhus occurring in the Federated Malay States the usual strains of "X 19" fail to detect agglutinins which are revealed by an anindologenes strain "Kingsbury."

A number of investigators seem to consider that by calling the Weil-Felix reaction a phenomenon of paragglutination and by rendering ordinary strains of *B. proteus* agglutinable by cultivating them in contact with the blood and body juices of patients or animals infected with the typhus virus, an explanation of the reaction is afforded, *e.g.* Papamarku (1918), Oettinger (1918), Grütz (1919), Silber (1923).

The fact that strains of "X 19" are very rarely found in the bodies of typhus fever patients and that Wolf (1922) has, both in Roumania and Berlin, isolated them from the excretions of persons who were not suffering from

typhus fever and who had not been in contact with the disease, renders such an explanation of the agglutinability of the bacilli unnecessary.

Moreover the paragglutination theory affords little or no explanation of what is the crux of the problem—the steady rise and decline of heterologous agglutinins in the typhus fever serum. Schaeffer's view (1919), that the Weil-Felix reaction is due to an increase of normal agglutinins not only for "X 19" but for various other bacteria is not very helpful as the origin of normal agglutinins has not been explained.

In recent years the view has been widely held that the virus of typhus fever is in some way associated with the *Rickettsia prowazeki* so that the statement of Krukowski (1924) that the blood serum and cerebro-spinal fluid of typhus fever patients agglutinate Proteus "0" "X 19" and also Rickettsia and that saturation with "0" "X 19" leaves the anti-Rickettsia agglutinins intact is of peculiar interest.

Abe (1924) explains the Weil-Felix reaction on the ground that there exist in *Rickettsia prowazeki* and in Proteus "0" "X 19" common antigen elements: like Breinl (1923) he finds that rabbits inoculated with *Rickettsia* or with typhus virus passed through the bodies of lice develop agglutinins for "X 19" earlier than when they are inoculated with the passage virus contained in the brain of infected guinea-pigs.

The view that the typhus virus unaided can cause the development of heterologous agglutinins would seem to be established in the case of rabbits where as Doerr and Pick (1919) noted not only may agglutinins for "X 19" be formed but also for *B. typhosus*. The difficulty of accepting this view is that agglutinins do not seem to be developed in the blood of the horse, sheep, dog and guinea-pig according to the results of Kraus and de la Barrera (1922).

Some remarkable views and experiments have been reported by Weigl (1923) and by Fejgin (1924) which if confirmed would go far not only to clear up the etiology of typhus fever but also to explain the Weil-Felix reaction. According to Weigl the bacillus of Plotz, the *Microbion typhi exenthematici* (Barykin), the As bacilli (Kuczynski) are variants of the typhus virus of which *Rickettsia prowazeki* would be the virulent form. Fejgin cultivated from the organs of typhus-virus-infected guinea-pigs seven strains of bacteria belonging to the Proteus group but fermenting no sugars and forming no indol, but agglutinated by "0" "X 19" and H "X 19" sera. Moreover Fejgin claims to have produced somewhat similar strains by acting on "X 19" with a specific bacteriophage and that inoculation of guinea-pigs with H "X 19" lysed cultures produced a reaction analogous to the malady of Nicolle. Passage experiments through a series of 14 to 20 animals were successfully carried out with the organs of guinea-pigs infected with the filtrates of the lysed cultures. Still further he claims that guinea-pigs which had first been inoculated with a typhus virus were refractory to infection with the bacteriophage and that conversely the bacteriophage anti-H "X 19" conferred on over 50 per cent. of guinea-pigs complete immunity to the typhus passage virus.

These results if confirmed would seem to establish the specificity of the "X 19" strains and thus explain the Weil-Felix reaction as one of ordinary immunity. Will it however explain the other agglutinins that may be found in typhus sera, *e.g.* for *B. typhosus*, *B. coli*, bacillus "U," *B. agglutinabilis* "U_2," *B. pyocyaneus*.

It was a consideration of this point that has led me to report in detail my experience with *B. agglutinabilis* "U_2."

Summary.

1. The development in typhus fever blood of agglutinins for a bacillus of the colon-paratyphoid group, which was isolated in 1921 from the faeces of a typhus case is shown to be a frequent occurrence in the disease as met with in Ireland, Poland and Syria. The bacillus has preserved its agglutinability up to the time of writing—a period of five years. Owing to its similarity to a bacillus previously isolated by the writer and which was designated bacillus "U," this bacillus has been named *B. agglutinabilis* "U_2."

2. It has been shown that bacteria preserved in alcohol even for 3 years are sensitive for use in the serological test, and that sera to which equal volumes of pure glycerine have been added retain their agglutinins for at least 5 years.

3. Five strains of *B. proteus* "X 19" fermented salicin with the production of acid and gas. Ordinary Proteus strains had no action on this glucoside and neither had the anindologenes strain "Kingsbury" although belonging to the "X 19" group.

4. Strains of *B. proteus* "X 19" show great differences in their sensitiveness to typhus fever agglutinins.

5. Views which have been advanced to explain the Weil-Felix reaction are discussed and the conclusion is reached that to label it as an instance of paragglutination does not account for the rise and fall of the agglutinins—agglutinins which are formed not only for *B. proteus* "X_2" and "X 19" but for certain strains of the *B. pyocyaneus* and colon-paratyphoid-typhoid groups.

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ADDENDUM.

Three additional observations which are confirmatory of the views and conclusions expressed in the foregoing paper may be given.

(1) A *B. proteus* XI 9 has been isolated by me from the stools of a patient in Enniskillen who was not suffering from typhus fever and who had not been in contact with such cases.

(2) The serum of a recent typhus fever case, 2. IV. 1927, occurring in Derry, agglutinated *B. agglutinabilis* "U_2" and our laboratory strain X 19 in dilutions of 1 in 640 and 1 in 1280 respectively, whereas its titre for our new *B. proteus* X 19 strain "Enniskillen" was 1 in 5120.

(3) Dr W. Fletcher has allowed me to use the following notes contained in a private communication:

(*a*) The blood sera of 21 controls in Kuala Lumpur who were not suffering from typhus did not agglutinate *B. agglutinabilis* "U_2" in dilutions above 1 in 60 except in one instance when it occurred at 1 in 120; this was a case of tsutsugamushi, a disease which is closely allied to typhus.

(*b*) *B. agglutinabilis* is agglutinated by the blood of persons with tropical typhus of the W group, but not as strongly as *B. proteus* X 19. It was agglutinated by a dilution of 1 in 4000 of a patient's serum collected on the twelfth day of illness. *B. proteus* X 19, strain Warsaw, was agglutinated at twice this titre.

(*c*) *B. agglutinabilis* is not agglutinated by the blood of patients with tropical typhus of the K group. It was not agglutinated above a titre of 1 in 60 by a patient's serum which agglutinated *B. proteus* strain Kingsbury, at 1 in 30,000 on the nineteenth day of illness.

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