SEROLOGICAL VARIETIES OF TYPHUS FEVER.

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I. Introduction.

To most bacteriologists the serology of typhus fever ceased to be an interesting problem when the diagnostic significance of the agglutination reaction with *B. proteus* X 19 had been generally recognised and the insignificance of this organism as the aetiological factor in typhus fever had been generally proclaimed. In recent years, however, a fresh interest has been aroused in view of the occurrence in endemic form of typhus or typhus-like fevers reported from various parts of the world, where their existence had hitherto not been established.

Fairly comprehensive reviews of these diseases have been published by Burnet and Durand (1929) and by Fletcher (1930). Out of the long list of these typhus-like fevers the following are the best known, since they have been the subject of numerous and valuable investigations: the endemic typhus (Brill's disease) of the United States of America (Maxcy and co-workers, 1923); the endemic typhus of Australia (Hone, 1922); the tropical typhus of the Federated Malay States (Fletcher and co-workers, 1925); the "fièvre exanthématique" of Marseilles (Olmer, 1925, and others).

The clinical symptoms of these diseases differ but little from those seen in mild cases of classical European typhus. Their epidemiology, however, is still obscure; there is no evidence of transmission by lice, no tendency for the disease to spread from man to man and the rôle of certain suspected parasites

is still hypothetical. The laboratory diagnosis is established by the agglutination reaction with *B. proteus* X 19, with the exception of the "fièvre exanthématique" of Marseilles, where this reaction is negative in the majority of cases.

In the endemic typhus of the United States of America and of Australia the agglutination reaction with X 19 occurs with the same frequency and uniformity as in the classical European form. Havens (1927) found the reaction positive in about 95 per cent. of cases in the United States of America, while in the series of cases reported from Australia (Hone, 1923; Bull, 1923; Wheatland, 1926) it was found positive in 100 per cent. In the Federated Malay States, however, Fletcher and his co-workers (1925–30) have established the important fact that the cases of endemic typhus occurring there, and called by them "tropical typhus," belong to two groups, distinctly different in their serum reactions: one group, occurring among the urban population, which reacts with the usual X 19 strains in the same way as cases of typhus fever in other parts of the world; and a second group of cases, occurring among the rural population, whose serum invariably fails to react with the usual X 19 strains, but reacts specifically with one particular strain of X 19, called the "Kingsbury" strain. Fletcher and Lesslar (1926) originally designated the urban group as "Group W" and the rural group as "Group K," according to the designation of the two cultures of *B. proteus* X 19 used in this differential diagnosis; these two cultures were named the "Warsaw" strain and the "Kingsbury" strain respectively. Recently, the name "shop-typhus" has been suggested for the urban (W) group and "scrub-typhus" for the rural (K) group, according to their different epidemiology (Fletcher, 1930).

It is obvious that the facts established by Fletcher and Lesslar are of the greatest importance for the practice of the serological diagnosis of typhus and typhus-like fevers. Moreover, they have a direct bearing on the intricate question of the relationship of *B. proteus* to this group of diseases. The following investigation was, therefore, undertaken with the view of testing the antigenic relationship between the so-called Kingsbury strain of *B. proteus* X 19 and genuine and variant strains of *B. proteus* X, known from the older work of Weil and Felix.

This investigation was made possible by the kind co-operation of Dr Fletcher and Dr Lewthwaite of the Kuala Lumpur Institute, who, during the last two years, supplied us with sera from a considerable number of patients suffering from the two types of tropical typhus and from tsutsugamushi disease occurring in the Federated Malay States. Our thanks are also due to Dr K. F. Maxcy of the United States Public Health Service, Washington; Dr J. Goldberzanka of the State Institute of Public Health, Warsaw; Prof. W. J. Wilson of the Queen's University, Belfast; Dr N. C. R. Keukenschrijver of Sumatra; Prof. R. Kawamura of the Niigata Medical College, Japan; Dr L. B. Bull of the Government Laboratory, Adelaide, Australia. We are glad of this opportunity of thanking all of them for their kindness in sending us sera from patients suffering from typhus and various typhus-like diseases.

II. THE TWO SEROLOGICAL TYPES OF TROPICAL TYPHUS (FLETCHER AND LESSLAR).

Fletcher and Lesslar (1925, 1926) published numerous and thoroughly observed curves of agglutinin formation in the serum of patients belonging to the two serological groups of tropical typhus. These curves demonstrated convincingly that the relationship between the "Kingsbury" strain and group K sera was as specific as that between the "Warsaw" strain and group W sera. No observation, however, was recorded by these workers to indicate the type of agglutination observed, whether due to O or H agglutinins.

In classical typhus the Weil-Felix reaction is invariably due to O agglutinins in the patient's serum as well as in the serum of animals infected with typhus virus (Weil and Felix, 1917 *b*, 1921). It appeared, therefore, necessary to test sera of the two types of tropical typhus with the view of excluding the possibility of H agglutinins being responsible for the divergence in reaction in the two groups. This seemed to be the more necessary since the cultures used by Fletcher and Lesslar, according to the description given by them, undoubtedly were H variants of *B. proteus* (motile bacilli, spreading film-like growth on agar). The result of this examination is shown in Table I.

Table I. *The two serological groups of tropical typhus in the Federated Malay States.*

	Case No.	Serum examined in London on	Blood drawn in Kuala Lumpur on	Day of illness	Titre of agglutination with strains					
					HX 19	OX 19	HX 2	OX 2	HXK	OXK
Group W = Urban group ("Shop-typhus")	1	3. iv. 29	1. iii. 29	?	2000	5000	0	0	0	0
	2	19. xi. 29	14. v. 29	?	1000	2000	100	100	0	0
	3	19. xi. 29	16. vii. 29	?	1000	2000	100	100	0	0
	4	11. ii. 30	27. xi. 29	?	2000	5000	0	0	0	0
Group K = Rural group ("Scrub-typhus")	1	26. vi. 28	25. v. 28	21	0	0	0	0	10,000	20,000
	2	26. vi. 28	18. v. 28	19	0	0	0	0	5,000	10,000
	3	9. ii. 29	2. i. 29	23	0	0	100	100	2,000	5,000
	4	9. ii. 29	11. vi. 28	22	0	0	0	0	5,000	10,000
	5	9. ii. 29	4. vii. 28	13	0	0	0	0	1,000	2,000
	6	3. iv. 29	?	?	0	0	0	0	1,000	2,000
	7	27. vi. 29	14. v. 29	?	0	0	0	0	1,000	2,000
	8	27. vi. 29	13. v. 29	?	0	0	0	0	500	1,000
	9	11. ii. 30	20. xi. 29	?	0	0	0	0	1,000	2,000
	10	11. ii. 30	20. xi. 29	?	0	0	0	0	2,000	5,000

Agglutination with fresh saline suspensions of living bacteria. Total volume, 1 c.c. Reading after 20 hours, 2 hours' incubation at 37° C., then at room temperature. Titre = highest dilution showing partial agglutination (estimated with the naked eye). Titre 0 = a negative result in dilution 1:100.

Sera from patients in the Federated Malay States, belonging to the two serological types of tropical typhus, have been tested against the H and O variants of the Kingsbury strain and of X 19 and X 2, the two serological types of *B. proteus* X strains known from the work of Weil and Felix. From a comparison of the agglutination titres for the corresponding pairs of O and H variants it is seen that the reactions are invariably due to O agglutinins, since—with the exception of type X 2—the O variants react in every instance up to a higher serum dilution than the corresponding H variants. It is further seen that the specificity of reaction established by Fletcher and Lesslar for

the two groups of tropical typhus is fully confirmed by the results tabulated in Table I.

From these two facts, viz. specificity and O type of the agglutination reaction, the conclusion has to be drawn that the so-called Kingsbury strain of *B. proteus* X plays the same rôle in the serum reactions of the cases in the rural group K as do the usual X 19 strains in the urban group W and in endemic or epidemic typhus in other parts of the world.

III. The history of the so-called K or Kingsbury strain of *B. proteus* X.

So far as the history of this strain could be ascertained it was supplied to the Bland-Sutton Institute in 1921 by the National Collection of Type Cultures as a typical strain of *B. proteus* X 19; and as such it was brought out to the Straits Settlements by Dr A. N. Kingsbury in 1923. This strain was the one employed by Fletcher and Lesslar (1925) in their earlier investigations, which led to the discovery of the typhus nature of the endemic fever of the Malay States now known as tropical typhus. Other strains of *B. proteus* X 19 were then obtained from laboratories in different countries and tested as a routine measure in agglutination tests with the result that the two serological groups K and W have been demonstrated.

Fletcher and Lesslar (1926) compared the Kingsbury strain with eight cultures of X 19, which they obtained from various sources, and found that it did not produce indol and did not ferment saccharose and maltose as all these other X 19 strains did; moreover, it showed certain differences in agglutination and absorption tests with rabbit immune sera, although these reactions proved that all the nine strains were related to one another. On the other hand, when they compared the K strain with fourteen cultures of van Loghem's anindologenes group of *B. proteus*, it was found to differ from them serologically, though it resembled them culturally; moreover, it was the only one of these non-indologenic strains which was agglutinated by the blood of persons suffering from tropical typhus. Fletcher and Lesslar concluded that the Kingsbury strain was neither an ordinary member of the indologenic nor of the non-indologenic group.

Wilson (1927) also compared the Kingsbury strain with other X 19 strains and confirmed the differences in the fermentation of saccharose and maltose and in the formation of indol. As regards serum reactions, however, his conclusion—based on some absorption tests—was, that in sera from typhus patients the agglutinins for "Kingsbury" and the X 19 strains were identical.

Since *B. proteus* is flagellated and possesses the two kinds of antigen known as the O and the H, it is obvious that only the qualitative method of serological analysis is capable of disclosing the true antigenic relationship between members of this group.

IV. THE ANTIGENIC RELATIONSHIP BETWEEN THE KINGSBURY STRAIN AND GENUINE STRAINS OF *B. PROTEUS* X.

Two serological types of *B. proteus* X strains have been known since the very beginning of the application of the agglutination reaction in typhus fever. The strains X 1 and X 2 were cultivated first (Weil and Felix, 1916) and were used in routine diagnosis during the epidemic of 1915–16. The agglutination with these strains reached modest titres only, 1:500 exceptionally being exceeded, but the reaction proved to be specific. During the course of that epidemic this organism was isolated a further sixteen times from the blood of typhus patients until, at the end of the epidemic, the nineteenth strain was cultivated, which is known as X 19 (Felix, 1916). The eighteen strains isolated first and named type X 2 were serologically identical, and appeared to differ from the type X 19 in one respect only: the latter reacted with the serum of typhus patients in very much higher dilutions than the former, the difference being ten-fold, hundred-fold and even more, while no difference could be established when the patient's serum was replaced by rabbits' sera homologous to either of these two types.

The true difference between the two types was not revealed until they were separated into their O and H variants (Weil and Felix, 1917 *b*). The O cultures OX 2 and OX 19 differ antigenically from each other not less than do the various types of meningococcus, pneumococcus or dysentery bacilli. Judged from the amount of common group agglutinins the recognised serological types of these latter species show even closer relationships than is the case with the two types of X strains. Rabbits, if intensively immunised with OX 19 or OX 2, either do not develop group agglutinins for the heterologous type at all or form very small quantities only, the difference in the titre being similar to that in the serum of typhus patients. Table II demonstrates the similarity in reaction of a patient's serum and an OX 19 immune rabbit serum.

Table II. *Group agglutinins of O type for X 2 in a patient's serum and a rabbit immune serum.*

Serum	Type of agglutination	Titre of agglutination with strains			
		HX19	OX19	HX2	OX2
Typhus patient (18th day of illness) from Adelaide, Australia	0	10,000	20,000	400	400
Rabbit immunised with OX19 (4 intravenous injections of OX19 heated for half an hour at 60° C.)	0	5,000	10,000	200	200

Moreover, if the usual technique of absorption tests is applied to such rabbits' sera, it is found that the small amount of group agglutinins is not absorbed or only very incompletely absorbed by the homologous organism, which had given rise to their production. An example is given in Table III.

Table III. *Absorption of group and main agglutinins from a patient's serum and a rabbit immune serum.*

Agglutination with strain	Serum of typhus patient from Australia: Absorbed in dilution 1:25 with OX19	Serum of typhus patient from Australia: Absorbed in dilution 1:25 with OX2	Serum of typhus patient from Australia: Unabsorbed	Serum of rabbit immunised with OX19: Absorbed in dilution 1:25 with OX19	Serum of rabbit immunised with OX19: Absorbed in dilution 1:25 with OX2	Serum of rabbit immunised with OX19: Unabsorbed
OX19	<1000	20,000	20,000	<500	10,000	10,000
OX2	400	<25	400	200	<25	200

In this respect, too, these O immune sera tally with the sera from typhus patients, but differ from those in the typhoid-paratyphoid group, where the group agglutinins are as readily absorbed by the homologous as by the heterologous organism. The fact that the agglutinins for X 19 and X 2 could not be removed by cross-absorption tests from human typhus sera, where they occur simultaneously, had been commented upon in numerous papers dealing with the theory of the X 19 reaction. Since Weil and Felix showed that rabbit O immune sera behave exactly in the same way, it became clear that the phenomenon is due to the very minute overlapping in O antigen between the two types. The difference in sensitiveness of the two reactions, viz. agglutinin absorption and agglutinin production, is the sole responsible factor.

It appeared, therefore, justifiable to consider X 2 and X 19 as two distinct serological types on the same grounds on which serological types have been recognised in various species. If it were not for the existence of the H antigen which is common to their H variants, the close relationship between these two organisms could not be demonstrated by serum reactions. Weil and Felix designated the *B. proteus* X strains as "specific" in contrast to *B. proteus vulgaris*, because they could not be recovered from sources unrelated to typhus. While varying degrees of community of the H antigen were established between the X strains and *B. proteus vulgaris*, the specific OX 19 and OX 2 antigens could not be found in any instance among the cultures of *B. proteus vulgaris* isolated from various sources, including those which existed in various collections prior to 1918 (Weil and Felix, 1918). On the other hand, the examination of twenty-four genuine strains of type X 2 and of thirty-one strains of type X 19, isolated by these and other workers in various parts of Europe and Asia Minor, showed for each type uniformity of antigenic composition.

The so-called "Kingsbury strain of X 19" was compared with six genuine strains of type X 2 and six genuine strains of type X 19 whose histories are known from the older work of Weil and Felix, Zeiss (1918) and others. Some of these strains were used in the form of their H variant only, while others, including the Kingsbury strain, were available in both the H and the O variants. Agglutination, agglutinin absorption and production of agglutinins in rabbits were applied in numerous experiments. It is not proposed to present

the results obtained in tabular form, since the technique of qualitative analysis of H and O antigens is now sufficiently well known.

It was found that the H antigen of the Kingsbury strain is, in part, identical with that common to all X strains, while its O antigen is completely different from both the OX 2 and OX 19 antigens.

With regard to the H antigen it must be stated that, in previous work on the *proteus* X strains, much less attention has been paid to this component than to the O antigen, since it has been recognised that it is the O antigen which separates the two types of X strains from each other as well as from all the types or races of *B. proteus vulgaris*. Such incomplete analysis as has been recorded suggested that the H antigen is uniform in both types of X strains, while its complexity was clearly disclosed as soon as cultures of *B. proteus vulgaris* were included in this analysis. Evidence of the existence of specific and group components in the H antigen of *B. proteus* strains, comparable to those known in the Salmonella group, has been published by Weil and Felix (1917 *a*, 1918).

Attention was directed chiefly to the analysis of the O antigen of the Kingsbury strain. In absorption tests with pure O rabbit immune sera the complete difference in the O antigens of each of the types X 19, X 2 and Kingsbury was clearly demonstrated. No trace of group agglutinins of the O type could be detected for the Kingsbury strain in the serum of numerous rabbits immunised with several genuine strains of both types X 19 and X 2 and *vice versa*, while the two types X 2 and X 19 produced in some animals group O agglutinins for each other reaching a titre similar to that demonstrated in Table II. In order to exclude the possibility that a supposed minute amount of group O antigen might be destroyed by prolonged exposure to 100° C. (Schütze, 1930), suspensions of O variants heated for half an hour at 58° C. were used for the immunisation of these rabbits, and suspensions of living bacteria were employed for agglutination and absorption tests. Still the result invariably obtained was that no community of O antigen could be established between the K culture and the two types of X strains.

It has been shown by Wassermann (1903) and Pfeiffer (1904) that various species of animals differ considerably in their antibody response to the injection of the same bacterial antigen. Thus the complex structure of an antigen may not be disclosed unless several species of animals are employed for the production of immune sera. Friedberger, Zorn and Meissner (1922), using chickens for this purpose, were able to demonstrate community in the O antigen of the X strains and the culture of *B. pyocyaneus* Z 1 which Neukirch and Kreuscher (1919) had found to be agglutinated by a certain proportion of cases of typhus fever. We, therefore, applied the same procedure and immunised chickens with the K strain as well as with X 19 and X 2. These sera, however, reacted in the same way as those of the corresponding rabbits which were immunised at the same time, *i.e.* no community of O antigen between the K strain and the two X strains was disclosed, although all the

chicken sera had a high O agglutination titre (1:10,000) for their respective homologous organisms.

From the results summarised above and from what is known from the older work on the antigenic relationship of the two types of X strains to each other and to *B. proteus vulgaris*, two alternatives offered themselves for the classification of the so-called "Kingsbury strain of X 19":

(*a*) This strain could be considered to be an ordinary *B. proteus vulgaris* belonging to Group III in the scheme of Weil and Felix (1917 *a*, 1918) and mistakenly labelled as X 19.

(*b*) It could be regarded as a member of the group of the so-called specific *proteus* X strains, representing a third serological type, whose O antigen differs entirely from those of the two types known hitherto, and does not show even that slight degree of relationship that is indicated by the poorly developed group antigen common to OX 2 and OX 19.

We favoured the second alternative, since it did not appear to us justifiable to neglect the actual history of this strain, and since its remarkably specific relationship to one particular group of typhus cases, established by Fletcher and Lesslar and confirmed by our present investigation, is unparalleled by any facts known from the rather eventful history of serological reactions in typhus fever. We, therefore, considered the Kingsbury strain to be a variant derived from the *B. proteus* X 19 which Dr Kingsbury originally obtained from the National Collection of Type Cultures. To test this hypothesis the Kingsbury strain was compared with variant strains of *B. proteus* X.

V. The antigenic relationship between the Kingsbury strain and variant strains of *B. proteus* X.

Antigenic variants of *B. proteus* X strains due to alteration of their O antigen have been described by Weil (1920, 1922) and by Felix (1922). Following the procedure of Baerthlein (1918) Weil succeeded in isolating from a single cell culture of OX 19 a variant strain, which antigenically differed completely from the parent strain and showed, by marked group agglutination with OX 2, a closer relationship to this type than to its parent strain. By analogy, X 2 was considered to be a variant of X 19. Felix confirmed and amplified this finding in a study of numerous variants derived from several genuine X strains of both types.

This antigenic variation is concerned with the O antigen only, like Arkwright's (1921) rough variation, with which it is in some way associated. As can be seen from the papers quoted above, numerous variants showing varying degrees of "roughness" have been encountered among the variants of *B. proteus* X; no attention, however, has been paid to the criteria of "roughness" known from the work of Arkwright and others, the sole interest being directed to the serological investigation of the variants.

The definition suggested for this kind of antigenic variation is: that it

leads to the complete loss of the original main O antigen of the parent organism and to the production of another entirely different O substance in the variant. By the isolation of intermediate forms it was shown that the antigenic variants are not formed suddenly but are the result of a gradual change in two directions, viz. the gradual destruction of the original antigenic substance, and the gradual formation of the new substance. The final products of this complex process, the antigenic or serological variants as previously defined, can only be classed as serological types, analogous to those occurring in nature and recognised in many bacterial species. Though the range of this variation is very wide and numerous variants with entirely different O antigens have been derived from the same parent culture as well as from other strains of the same type, still the differentiation between *B. proteus* X and *B. proteus vulgaris* remains definite, and by O agglutination the latter group can be as sharply distinguished from the variants as from the genuine X strains.

Ten of these variants which had survived in agar stabs eight years were available for comparison with the Kingsbury strain. Seven had been derived from four genuine strains of the X 19 type, while three originated from three genuine cultures of the X 2 type. The re-examination of these variants showed that they had maintained their antigenic properties exactly as they had been described originally (Felix, 1922). However, when tested for "roughness" by Arkwright's technique (in saline solutions of increasing salt concentration) varying degrees of roughness were disclosed as is seen from Table IV. This table also shows the reaction of the ten variants tested with pure O sera of rabbits immunised with OX 19, OX 2 and the Kingsbury strain, respectively.

Table IV. *Serum and salt agglutination of variant strains of* B. proteus *X.*

Agglutination with variant strain	Type of parent strain	Serum agglutination (in normal saline). Dilution 1:200 of O serum of rabbits immunised with strains			Salt agglutination NaCl (%)		
		Kingsbury	X 19	X 2	0·85	1·7	3·4
IR	X 19	±	+ + +	+ + +	±	+ + +	+ + +
H III	,,	−	+ + +	+ + +	−	±	+ +
OX 19 b 4	,,	−	+ + +	+ + +	−	+ +	+ + +
OS 1, No. 1	,,	+ + +	−	−	−	±	+
S 1, Tr. 2	,,	−	−	±	−	−	+
33,180, No. 3	,,	±	±	±	±	+ +	+ + +
X 21, No. 3	,,	+ + +	−	−	−	±	+
OX 2, No. 4	X 2	−	−	+ + +	−	±	±
311, No. 3	,,	−	−	−	−	−	−
42,428, No. 3	,,	−	−	−	−	−	−
Strain				Controls			
Kingsbury		+ + +	−	−	−	−	−
X 19		−	+ + +	−	−	−	−
X 2		−	−	+ + +	−	−	−

The variants are here denoted by the same symbols which were used in the original description (Felix, 1922). Some of them are agglutinated weakly or strongly by the serum corresponding to the type of their parent, while others do not react at all. Some are disclosed as "rough" by normal saline, others

only by salt solutions of higher concentration. The most interesting results, however, are those under the heading of the Kingsbury serum: two out of the ten variants tested are agglutinated by this serum, viz. OS 1, No. 1, and X 21, No. 3.

The former is a descendant of a genuine type X 19 strain, isolated by Zeiss (1918) in Smyrna and designated S 1; the latter is derived from a genuine type X 19 strain isolated by Weil in Poland (1917) and designated X 21.

Since both these variants, because of their agglutinability by 1·7 per cent. salt solution, are characterised as rough, and serum agglutination with suspensions of rough organisms is known to be liable to many pitfalls, some attempts have been made to isolate corresponding smooth variants. These attempts, however, remained unsuccessful. The following detailed analysis of these two variants and the Kingsbury strain was, therefore, conducted in such a way as to employ the variants chiefly for the production and absorption of agglutinins, while agglutination tests were mostly set up against the Kingsbury strain, whose "smoothness" throughout the course of this investigation remained as perfect as that of all the genuine X strains.

Rabbits were immunised by intravenous injections of suspensions of the two variants OS 1, No. 1, and X 21, No. 3, and their parent strains S 1 and X 21. Since the latter were available as H variants only, suspensions heated to 100° C. for 2 hours had to be used in order to obtain pure O sera. Both variants produced in the greater number of rabbits immunised, marked group O agglutinins for the Kingsbury strain, while their respective parent strains entirely failed to do so, like all the other genuine X strains which had already been tested with invariably negative results, as stated in the preceding section.

Table V. *Group O agglutinins for the Kingsbury strain in the serum of rabbits immunised with* B. proteus *X variants.*

Serum of rabbits before and after immunisation with suspensions heated at 100° C. of strain

Agglutination with the H form of strain	Serum dilution	Rabbit 265 Normal	Rabbit 265 Variant OS 1, No. 1	Rabbit 274 Normal	Rabbit 274 Variant OS 1, No. 1	Rabbit 278 Normal	Rabbit 278 Variant X 21, No. 3	Rabbit 269 Normal	Rabbit 269 Genuine S 1	Rabbit 270 Normal	Rabbit 270 Genuine X 21	Controls NaCl (%) 0·85	1·7	3·4
S 1	1:25	–	–	–	–	–	–	–	+++	–	+++	.	.	.
	1:50	–	–	–	–	–	–	–	+++	–	+++	–	–	–
	1:100	–	–	–	–	–	–	–	+++	–	+++	.	.	.
	1:200	–	–	–	–	–	–	–	+++	–	+++	.	.	.
X 2	1:25	–	–	–	±	–	+±	–	++±	–	–	.	.	.
	1:50	–	–	–	–	–	±	–	+±	–	–	–	–	–
	1:100	–	–	–	–	–	–	–	+	–	–	.	.	.
	1:200	–	–	–	–	–	–	–	–	–	–	.	.	.
Kingsbury	1:25	–	+++	–	++±	–	++±	±	±	–	–	.	.	.
	1:50	–	++	–	+±	–	+±	–	–	–	–	–	–	–
	1:100	–	+±	–	±	–	±	–	–	–	–	.	.	.
	1:200	–	±	–	–	–	–	–	–	–	–	.	.	.
Titre for homologous strain		.	1:2000	.	1:2000	.	1:5000	.	1:10,000	.	1:5000	.	.	.

+++, ++, +, ± = varying degrees of agglutination, estimated with the naked eye.
The degree of agglutination indicating the "titre" in all the other tables corresponds to the sign +.

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Table V illustrates some of the results obtained in these rather numerous experiments. It is seen that the variant sera possess relatively high titre group agglutinins for the strain Kingsbury, whereas they have none for the strain S 1, which is the parent strain of one of these variants. The other parent culture, X 21, was also tested along with the usual X 19 culture; since the results obtained were identical with those for S 1 they are omitted from the table. Neither of the parent strains produced any trace of group agglutinins for the Kingsbury culture (rabbits 269 and 270).

It has been shown in the previous section that the amount of group O antigen common to the two types of *B. proteus* X strains is very much smaller than that known in many other species, particularly among the members of the Salmonella group. If this well-established fact is taken into consideration the results demonstrated in Table V can only be interpreted as indicating definite antigenic relationship between the Kingsbury strain and the two variants. Judged from the incidence and the titre of group O agglutinins produced in the rabbit, this relationship is even closer than that between the types X 2 and X 19, and still more so when the variants are compared with their parent cultures.

The objection might be raised that the "roughness" of the two variants was responsible for the community of O antigen established, since the view is held by some workers that rough variants possess a "cosmopolitan" antigen (Schütze, 1921). This objection, indeed, would carry little weight with the Kingsbury strain, whose perfect smoothness was always thoroughly checked. Nevertheless, the following control was added: rabbits were immunised with the variants H III and 33,180, No. 3, whose roughness is equal to or even greater than that of variants OS 1, No. 1 and X 21, No. 3 (see Table IV) and no trace of group O agglutination with the Kingsbury strain could be detected in these sera.

The relatively high degree of community in O antigen established by the experiments described above suggested the possibility of demonstrating it even by absorption tests. It has been shown in a previous section that this procedure is unsuccessful with the types X 2 and X 19 (see Table III). In the case of the Kingsbury strain and the two variants, however, it proved satisfactory beyond expectation.

Table VI. *Absorption tests with Malayan patients' sera of the K type.*

Serum from typhus patient of the K group	Titre of agglutination with strain Kingsbury. Serum dilution 1:200 absorbed with suspensions of living bacilli of strains								
	Kingsbury	Variant OS 1, No. 1	Variant X 21, No. 3	S 1 genuine	X 21 genuine	HX 19	OX 19	HX 2	Serum unabsorbed
Case 1	<200	<1000	<200	10,000	10,000	10,000	10,000	10,000	10,000
Case 2	<200	< 500	<200	5,000	5,000	5,000	5,000	5,000	5,000

In Table VI absorption tests with two sera from Malayan patients suffering from the K type of tropical typhus are reproduced. It is seen that the

Kingsbury agglutinins are absorbed completely or very markedly by the two variants but are left entirely intact by the genuine X cultures, including the two parent strains. Most of the K type sera specified in Table I were tested repeatedly in similar experiments, and other variant and genuine X strains were used as absorbents. Special attention was also paid to controls with the two rough variants H III and 33,180, No. 3, but no interaction with a supposed "cosmopolitan" rough antigen could be traced. The invariable result of these numerous absorption tests was in complete agreement with that obtained in the experiments on the production of group O agglutinins in rabbits, and indicated that the community in O antigen between the Kingsbury strain and the variants OS 1, No. 1, and X 21, No. 3, was indeed one of high degree.

These findings, in our opinion, strongly support the hypothesis put forward in the previous section, according to which the Kingsbury strain is a variant derived from the X 19 culture originally obtained from the National Collection of Type Cultures and represents another type of *B. proteus* X. We, therefore, propose to designate this type as *B. proteus* XK and to use the symbols HXK and OXK for its H and O variants, respectively.

VI. Cultural and biochemical properties of *B. proteus* X strains.

Little attention was originally paid to the biochemical properties of *B. proteus* X strains, since it was known already that in the *B. proteus* group (as a whole) these characters possess only a low degree of constancy. So far as they have been tested in this direction the following marked differences have been established among genuine strains of the two types X 2 and X 19: some strains invariably failed to liquefy gelatine or coagulated serum, while others did so constantly; the reaction in litmus-milk varied; on agar plates some strains never showed colonies other than those of the spreading film-like H type, whereas others regularly yielded a certain proportion of non-spreading O type colonies (Felix, 1922). Differences of the magnitude described in the case of the Kingsbury strain (Fletcher and Lesslar, 1926; Wilson, 1927) had, however, not been observed between various genuine strains of *B. proteus* X, while variant strains had not been tested in this respect.

Jötten (1919) and Schaeffer (1919) who first compared the cultural and biochemical properties of *B. proteus* X and *B. proteus vulgaris* found that indologenic strains almost invariably fermented maltose and saccharose, whereas the non-indologenic strains did not. The coincidence of these properties has been confirmed by other workers, although exceptions to this rule have also been reported (Wolff, 1922 *a*).

Now the objection might be raised that the difference in the ability to produce indol and to ferment maltose and saccharose established between the Kingsbury culture and *B. proteus* X strains is irreconcilable with our conclusion that the former is a variant derived from the latter. However, in the light of the present knowledge of bacterial variability this objection would carry but

little weight. No special effort therefore was made to isolate a non-indologenic variant from an indologenic parent X strain in order to meet such an objection. Wolff (1922 *a*), working with a strain of *B. proteus vulgaris* which produced indol and fermented maltose and saccharose, observed the complete loss of these properties when the strain was re-examined one year later. The strain had only rarely been subcultured during this time and, so far as the inadequate serological tests indicated, no correlated alteration in antigenic structure was established in this variant. Instances of variation in the production of indol and in other forms of decomposition of proteins have been described in various species (for references see Arkwright, 1930).

VII. OCCURRENCE OF AGGLUTININS FOR VARIOUS TYPES OF X STRAINS IN CASES OF TYPHUS FEVER FROM VARIOUS PARTS OF THE WORLD.

Since the superiority for diagnostic purposes of the type X 19 over the type X 2 has been recognised, the latter has fallen into disuse and the presence of agglutinins for type X 2 has consequently not been tested in cases of typhus fever occurring during various epidemics or in various parts of the world.

In a series of 225 typhus cases examined by Weil and Felix during the epidemic of 1915–16 in Eastern Galicia, only two cases (less than 1 per cent.) were encountered whose serum was devoid of agglutinins for X 2 throughout the whole course of the disease. Similar results were obtained by other workers during that epidemic (Salubritäts-Kommission, 1916). In the following year, however, in a series of 300 typhus cases from Asia Minor, 22 per cent. of cases were negative to type X 2, while the reaction with X 19 was positive in 100 per cent. of cases (Felix, 1917). Since then no adequate observations have been reported.

When the wide range of antigenic variability of *B. proteus* X was established, it was suggested as possible that this might invalidate the serological diagnosis of typhus cases (Felix, 1922). If the antigen which the typhus virus possesses in common with the X strains were, in the infected human body or in the vector, capable of variation to a similar extent as that established with the X strains *in vitro*, irregularity in the serum reaction of typhus patients had to be expected. In a few tests with some of the variants described and with a small number of patients' sera from Poland no indication of such a complication was found at that time. It now appears, however, to have been clearly demonstrated by the two serological types of tropical typhus described by Fletcher and Lesslar.

We have tested the sera of typhus cases from various parts of the world against the three types, X 19, X 2 and XK. The results are shown in Table VII.

It is seen that, irrespective of the origin of the sera, of the height of the agglutination titre with X 19 and of the presence or absence of group agglutinins for X 2, the reaction to type XK is invariably negative. In this respect these sera tally with the serum from cases of Fletcher and Lesslar's group W

of tropical typhus, and also with the serum of rabbits immunised with types X 19 and X 2 which have been shown to be devoid of group O agglutinins for type XK.

Table VII. *Agglutination of various types of X strains by sera from cases of typhus fever from various localities.*

Typhus patients from	Case No.	Serum examined in London on	Blood drawn on	Titre of agglutination with strains					
				HX19	OX19	HX2	OX2	HXK	OXK
United States of America	1	3. v. 28	10. iii. 27	200	500	0	0	0	0
	2	,,	8. vi. 27	500	1,000	0	0	0	0
	3	,,	12. xi. 27	200	500	0	0	0	0
	4	3. iii. 30	10. iv. 29	2,000	5,000	0	0	0	0
	5	,,	23. v. 29	1,000	2,000	0	0	0	0
	6	,,	10. vi. 29	500	1,000	0	0	0	0
	7	,,	20. vii. 29	2,000	5,000	0	0	0	0
Poland	1	25. iii. 30	6. iii. 30	2,000	5,000	200	200	0	0
	2	,,	,,	2,000	5,000	100	100	0	0
	3	,,	13. iii. 30	200	500	100	100	0	0
	4	28. v. 30	25. iii. 30	5,000	10,000	500	500	0	0
	5	,,	26. iii. 30	2,000	5,000	0	0	0	0
	6	,,	1. iv. 30	200	500	0	0	0	0
	7	,,	4. iv. 30	500	1,000	0	0	0	0
	8	,,	5. iv. 30	1,000	2,000	100	100	0	0
	9	,,	10. iv. 30	1,000	2,000	100	100	0	0
	10	,,	11. iv. 30	2,000	5,000	100	100	0	0
Ireland	1	12. i. 29	?	2,000	5,000	0	0	0	0
Australia	1	12. i. 29	2. viii. 28	10,000	20,000	400	400	0	0

With regard to the occurrence of group O agglutinins for type X 2, there appears to exist a difference between typhus cases from the United States of America and those of other origin. Most of the sera of typhus cases from Poland specified in Table VII contain significant group agglutinins for X 2, indicating that the agglutinogenic properties of the typhus virus in Poland have remained much the same as they were in 1915–16. Sera from group W cases from the Federated Malay States (see Table I) and the one serum from Australia which we have had an opportunity to examine, react like the sera from Poland. The American sera, however, do not contain any group agglutinins for X 2, and a similar result has been published recently by Spencer and Maxcy (1930). Should this finding be confirmed by investigations on a larger scale, another peculiarity of the virus of endemic typhus of the United States would thus have been established, in addition to the property of producing conspicuous scrotal and testicular lesions in infected animals, which this virus (Maxcy, 1929 *a*, *b*, *c*; Pinkerton, 1929) shares with those of the Mexican Tabardillo (Neill, 1917; Mooser, 1928, 1929) and of Rocky Mountain spotted fever.

Two details of a more technical nature must be mentioned in passing. O agglutination generally gives a distinctly higher titre with the O variant than with the corresponding H variant. This is the rule in *B. proteus* (Weil and Felix, 1917 *b*) as well as in the typhoid-paratyphoid group of organisms (Felix, 1930). With the variants of X 19 and XK this was consistently the

case throughout the present investigation. The culture OX 2, however, only yielded titres equal to or even lower than those for HX 2. The reason for this anomaly has not been ascertained. The other anomaly noted was that suspensions of both HX 2 and OX 2 were agglutinated by glycerole highly diluted with normal saline. A number of sera from typhus patients from America could not be included in Table VII, since their agglutination with HX 2 and OX 2 was evidently due to the non-specific action of glycerole which had been used as preservative. All the patients' sera tested in the course of this investigation had been neither inactivated by heat nor preserved by any disinfectant.

VIII. H AGGLUTININS AS SOURCE OF ERROR IN THE DIAGNOSIS OF TYPHUS AND TYPHUS-LIKE DISEASES.

The most important source of error in the Weil-Felix reaction is H agglutination with *B. proteus* X strains, since this type of agglutination is of no significance in the diagnosis of typhus (for references see Wolff, 1922*b*). H agglutinins due to an existing or a previous infection with *B. proteus vulgaris* (Groups II and III of Weil and Felix, 1918), such as cystitis, otitis, etc., are occasionally met with in the serum of healthy individuals or of patients suffering from various non-typhus diseases. Unless due attention is paid to the differentiation between H and O agglutinins instances of erroneous typhus diagnosis will necessarily occur and may lead to fallacious conclusions with regard to the occurrence and significance of agglutinins for various types of X strains. The case of "typhus-like" fever recently published from Australia (Penfold and Corkill, 1928) may serve as an example of the misleading effect of this source of error. The serum reactions of this patient are illustrated in Table VIII together with those of two cases from other localities.

Table VIII. *H agglutinins for* B. proteus *X strains in human sera.*

Case	Patient from	Serum examined in London on	Blood drawn on	Type of agglutination	Titre of agglutination with strains					
					HX 19	OX 19	HX 2	OX 2	HXK	OXK
1	Melbourne, Australia	15. ii. 29	21. iv. 28	O	0	0	0	0	0	0
				H	500	0	500	0	500	0
2	Kuala Lumpur, Federated Malay States	15. ii. 29	3 xi. 28	O	0	0	0	0	0	0
				H	2000	0	2000	0	100	0
3	Warsaw, Poland	25. iii. 30	14. iii. 30	O	2000	5000	0	0	0	0
				H	1000	0	1000	0	200	0

It is seen that the Australian serum, which we received through the kindness of Prof. Wilson, Belfast, contained H agglutinins only, *i.e.* its Weil-Felix reaction was negative. The same was the case with serum No. 2, which was sent to us with the diagnosis of tropical typhus of type W. Serum No. 3, however, from a typhus patient from Poland, gave O agglutination with type X 19, significant for typhus fever, in addition to H agglutination with all three types. It has been mentioned in Section IV that in *B. proteus* the H antigen is of a complex structure and that the Kingsbury strain and types

X 2 and X 19 possess only partly identical H antigens. This finds its expression also in the H titres recorded in Table VIII, which are equally high for all three types in serum No. 1, but differ markedly in the others.

IX. Serum reactions in cases of tsutsugamushi disease from the East Indies and Japan.

Cases of tsutsugamushi, the endemic typhus-like disease of Japan, are reported to give no reaction with X 19 (Ishiwara and Ogata, 1923; Kawamura, 1926). In the Malayan form of the disease the same result has been obtained with X 19, but with the Kingsbury strain agglutination in low serum dilutions has been observed (Fletcher and Field, 1927; Fletcher, Lesslar and Lewthwaite, 1929). With the kind permission of Dr W. Fletcher the details of his and his co-workers' observations are given in Table IX.

Table IX. *Agglutination of strain Kingsbury in Fletcher and his co-workers' cases of tsutsugamushi in the Malay States.* (*Personal communication from Dr W. Fletcher.*)

Case no. 1	Day of illness	10	12	14	—	—	—	—	—	—
(European)	Titre for XK	0	80	40	—	—	—	—	—	—
Case no. 2	Day of illness	10	12	22	25	28	35	—	—	—
(European)	Titre for XK	0	60	120	80	60	60	—	—	—
Case no. 3	Day of illness	3	10	15	20	24	34	41	—	—
(European)	Titre for XK	0	10	60	240	240*	100	60	—	—
Case no. 4	Day of illness	6	13	15	18	22	25	30	40	60
(European)	Titre for XK	0	60	60	60	60	60	90	120	30
Case no. 5	Day of illness	10	16	19	23	26	34	—	—	—
(Indian)	Titre for XK	60	240	240	240	120	60	—	—	—
Case no. 6	Day of illness	11	17	—	—	—	—	—	—	—
(European)	Titre for XK	60	60	—	—	—	—	—	—	—
Case no. 7	Day of illness	10	16	19	23	26	34	—	—	—
(Indian)	Titre for XK	160	240	240†	240	120	60	—	—	—

* Partial 640. † Partial 480.

Although the titres of the agglutination recorded in these cases are incomparably lower than those known from type K of tropical typhus, still their rise and fall is in several instances regular and distinct. Fletcher and his co-workers did not observe similar agglutination reactions with type XK in control tests with the blood of normal persons or of those suffering from other diseases. They, therefore, ascribed significance to these reactions in spite of the low titres reached and concluded that the two diseases are closely related.

We have tested sera from cases of tsutsugamushi disease from Sumatra and Japan. The results are shown in Table X.

When the results shown in Table X are compared with those recorded in Tables I and VII the conclusion drawn by Fletcher and his co-workers with regard to the relationship between tsutsugamushi and the K type of tropical typhus appears to be well justified. None of the tsutsugamushi sera reacted with the types X 19 or X 2, while the majority gave O agglutination with type XK. The Sumatran sera, Nos. 2 and 10, showing agglutination titres as

high as those met with in tropical typhus of the K type, may possibly belong to cases of this disease which have mistakenly been diagnosed as cases of tsutsugamushi. Both the W and the K type of tropical typhus are reported to occur in Sumatra (Wolff, 1929) and in Java (van Steenis, 1929; Peverelli, 1930) and the clinical differentiation between these diseases and tsutsugamushi is sometimes difficult. Unfortunately, we have been unable to secure the histories of the Sumatran cases.

Table X. *Agglutination of various types of X strains by sera from cases of tsutsugamushi disease.*

Tsutsugamushi patients from	Case no.	Serum examined in London on	Blood drawn on	Titre of agglutination with strains					
				HX19	OX19	HX2	OX2	HXK	OXK
Sumatra	1	29. i. 29	18. ix. 28	0	0	0	0	0	0
	2	,,	21. ix. 28	0	0	0	0	1000	2000
	3	,,	,,	0	0	0	0	200	500
	4	,,	24. ix. 28	0	0	0	0	0	0
	5	,,	,,	0	0	0	0	0	0
	6	,,	5. x. 28	0	0	0	0	100	200
	7	,,	,,	0	0	0	0	100	200
	8	,,	9. x. 28	0	0	0	0	0	0
	9	25. vi. 29	23. v. 29	0	0	0	0	0	0
	10	,,	,,	0	0	0	0	500	1000
Japan	1	11. ii. 30	29. ix. 29	0	0	0	0	100	200
	2	,,	30. x. 29	0	0	0	0	100	200
	3	,,	28. xi. 29	0	0	0	0	200	500

All the Japanese cases, whose selection we owe to the expert knowledge of Prof. Kawamura (Niigata, Japan) and the Sumatran cases, Nos. 3, 6, 7, tally with regard to their titre for XK with Fletcher's figures recorded in Table IX. The incidence of these agglutinins and their low titre strikingly recall the agglutination of *B. proteus* X 2 with typhus sera from Poland (see Table VII). Unless this analogy is entirely misleading the suggestion appears to be justifiable that the agglutination of type XK in tsutsugamushi is of the same order as that of type X 2 in typhus fever from Poland, *i.e.* group agglutination due to group O antigen which the K type of *B. proteus* X possesses in common with the unknown virus of tsutsugamushi disease. If this hypothesis were proved by the isolation from cases of tsutsugamushi of another serological type of *B. proteus* X corresponding to this virus, the analogy to the history of the X 19 reaction in European typhus would become complete. There, as is known, the group agglutination with type X 2 was found first and led to the discovery of the main agglutination with type X 19.

It appears not unlikely that further serological varieties of typhus may yet be recognised among the different typhus-like diseases in different parts of the world (Megaw, 1921, 1924; Burnet and Durand, 1929; Fletcher, 1930). Recent observations on Rocky Mountain spotted fever and on the "fièvre exanthématique" of Marseilles seem to lend support to this view.

X. Review of data published on serum reactions in Rocky Mountain spotted fever and in the "fièvre exanthématique" of Marseilles.

We have had no opportunity of testing sera from cases of these two forms of typhus-like diseases. A brief account of the serological data published by various workers may, therefore, serve to complete the survey which we are attempting.

(*a*) *Rocky Mountain spotted fever.*

Cases of this disease were first reported to give a negative reaction with X 19 (Kelly, 1923). Kuczynski (1927), however, found that the virus of Rocky Mountain fever produced X 19 agglutinins in infected animals and this result was confirmed by Otto (1928), Munter (1928) and Kerlee and Spencer (1929). The last mentioned workers and Spencer and Maxcy (1930) also established that the reaction was positive in a large proportion of human cases of the disease.

In cross-immunity tests with rats (Kuczynski, 1927) and with rabbits (Munter, 1928), it has been shown that the antigens responsible for the production of X 19 agglutinins are not identical in the viruses of typhus and Rocky Mountain fever, but possess an overlapping component in common. The observations made by the American workers point clearly to the same conclusion.

Kerlee and Spencer (1929) tested a few sera from human cases and found agglutinins for both the X 19 and the XK type together in one serum and separately in others. In their experiments with rabbits the infection with the virus of Rocky Mountain fever led to the production of agglutinins for both types and there was not much difference in the titres recorded for the two strains. Spencer and Maxcy (1930) recently published a comparative study of the agglutination reactions of human cases of Rocky Mountain fever and of endemic typhus of the United States. Sera from forty and sixteen cases of the two diseases, respectively, were tested against the O and H variants of the three types X 19, X 2 and XK. The difference in reaction in the two series was very conspicuous. In endemic typhus it was invariably the type X 19 which reacted with a high titre of agglutination, whereas significant group agglutinins for the two other types were absent. This is in agreement with our own experience (see Table VII). With Rocky Mountain fever, however, there was no uniformity of results: type X 19 reacted significantly in a minority of cases only and the titres of this reaction were markedly lower than those recorded in endemic typhus; moreover, agglutinins for types X 2 and XK were found in a considerable proportion of these cases. Even if allowance be made, in the case of strains HX 2 and OX 2, for the possible non-specific agglutination by glycerole which was probably present in some of the sera used, still the difference in reaction in the two series was striking. Spencer and Maxcy rightly concluded that there was a qualitative difference.

It would appear that two alternatives may be consistent with the established facts, viz. (1) the virus of Rocky Mountain fever may not be uniform

antigenically but may be divided into serological varieties similar to those established by Fletcher and Lesslar in Malayan tropical typhus; (2) the main O antigen of the virus may correspond to a type of *B. proteus* X other than the three types known hitherto, and the irregular reactions which these types give with sera from human cases of the disease may be due to common group O antigens.

(*b*) "*Fièvre exanthématique*" *of Marseilles.*

Numerous observations on this typhus-like disease have been published since D. Olmer (1925) first called attention to its occurrence in the Marseilles area. The disease is identified by some workers with the "fièvre boutonneuse" of Tunis, with the "febbre eruttiva" of Italy and with similar fevers occurring in other parts of the Mediterranean (for references see Conseil, 1929). The relationship of this disease to classical typhus is a matter of controversy, chiefly based on the negative results of cross-immunity tests and on the irregular agglutination reactions recorded by various workers. Samples of the same serum when tested in various laboratories against strains of *B. proteus* X have in some instances been recorded as positive by one worker and negative by another.

Burnet and Olmer (1927) published some results of the agglutination reactions obtained in nine cases of the Marseilles fever. Several strains of type X 19 and one of type X 2 were employed and in three out of the nine sera tested the reaction was considered as positive. The titres recorded were low (maximum 1:500) and the agglutination occurred in two cases with both types, and in one case with the type X 19 only. Burnet and Olmer (1927) state that, in an earlier series of forty-two cases observed by Olmer, the Weil-Felix reaction was negative, while Conseil (1929) makes the statement that the reaction was positive in nine out of forty-four of Olmer's cases. Further observations led Olmer (1928) to the conclusion that although some of the cases give a positive Weil-Felix reaction, this reaction is inconstant and quite unlike the results obtained in true typhus. Boinet, Piéri and Dunan (1928 *a*, *b*) had negative results only with both the X 19 and the XK type.

Assuming that the positive reactions referred to have not been due to some source of error, like H agglutinins in the patient's serum or "roughness" of the cultures used, then the most justifiable interpretation would seem to be that the irregular and low titre reactions with *B. proteus* X strains are group agglutinations due to the agglutinogenic properties of the unknown virus of the "fièvre exanthématique." Conseil (1929), however, states that these observations are of no significance since a positive Weil-Felix reaction is often met with in human sera during convalescence after various non-typhus diseases. This unwarranted statement is in sharp contrast to the unanimous opinion of those who have used the test extensively (for references see Otto and Munter, 1930). If the observations published by Burnet and Olmer (1927) were confirmed by adequately controlled tests with H and O variants of

B. proteus X they would, in our opinion, strongly suggest that "fièvre exanthématique" is another serological variety of typhus fever.

XI. The serum reactions of various forms of typhus and typhus-like diseases.

The list of various forms of typhus and typhus-like diseases, whose serum reactions are shown in Table XI, does not include a number of typhus-like fevers reported from various localities but not yet adequately studied. The reactions with *B. proteus* X 19 and XK only are specified in this table because type X 2 has not yet been shown to correspond to the main O antigen of the virus of any serological variety of typhus.

Table XI. *Showing the serum reactions of various forms of typhus and typhus-like diseases.*

Name of disease	Locality	Vector	Agglutination with *B. proteus* X	
			Type X 19	Type XK
Typhus (epidemic and endemic)	Old and New Worlds	Lice	Positive	Negative
Tabardillo (Mexican typhus)	Mexico	,,	,,	Not tested
Endemic typhus (Brill's disease)	South Eastern United States	Unknown	,,	Negative
Endemic typhus	Australia	,,	,,	,,
,,	Rome	,,	,,	Not tested
Rocky Mountain spotted fever	Western United States	Ticks	Partly positive	Partly positive
Tropical typhus, Type W	Malaya	Unknown	Positive	Negative
,, ,, K	,,	,,	Negative	Positive
Tsutsugamushi	Malaya and Sumatra	Mites	,,	Weakly positive
,,	Japan	,,	,,	,,
Typhus-like fever	Central India	Unknown	,,	Not tested
Fièvre exanthématique	Marseilles	,,	Negative (partly positive)	,,

It is obvious that this newer knowledge of serological types in typhus fever has a direct bearing on the practice of serological diagnosis. The evidence of unity among the different forms of typhus hitherto derived from the X 19 reaction is no longer valid. Serological types of the disease may exist whose diagnosis by agglutination will only become possible after the isolation of the corresponding types of *B. proteus* X. Cases of typhus-like diseases as those from the Marseilles district, from Central India (Megaw, Shettle and Roy, 1925), etc. may, therefore, prove to belong to the typhus group, although the X 19 reaction was found negative in these cases.

It is further obvious that the existence of serological varieties of typhus also invalidates the significance hitherto ascribed to negative cross-immunity experiments. Failure to obtain cross-protection between classical typhus and some typhus-like diseases may be due to differences in antigen such as occur in the serological types of bacteria. The typhus nature of a disease which otherwise is typhus-like should, therefore, not be definitely excluded on the basis of negative cross-immunity tests.

XII. SUMMARY.

1. Fletcher and Lesslar's observations on two serological types of tropical typhus have been fully confirmed.

2. The antigenic relationship between the indologenic *B. proteus* X 19 and the non-indologenic Kingsbury strain is of the same order as that obtaining between the X 19 and X 2 types of *B. proteus* X.

3. The Kingsbury strain is an antigenic variant derived from the original X 19 culture and represents another serological type of *B. proteus* X. The symbol XK is suggested for this type.

4. Sera from cases of classical European typhus and of endemic typhus of the United States of America and of Australia have been tested for the occurrence of main and group O agglutinins for the known types of *B. proteus* X.

5. H agglutination as source of error in the diagnosis of typhus cases is illustrated by some examples.

6. Sera from cases of tsutsugamushi from Sumatra and Japan react with type XK like the Malayan cases of this disease described by Fletcher and co-workers.

7. This latter reaction is of the order of group O agglutination. It is suggested that antigenically the virus of tsutsugamushi corresponds to another serological type of *B. proteus* X which is yet unknown.

8. The data published on the serum reactions in Rocky Mountain spotted fever and in the "fièvre exanthématique" of Marseilles are analysed. It is suggested that these two diseases represent further serological varieties of typhus.

9. The significance hitherto attached to negative agglutination tests with *B. proteus* X and to negative cross-immunity tests obtained with some typhus-like diseases requires revision in the light of recent observations.

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