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TYPHUS RICKETTSIAL AGGLUTINATION TESTS IN THE MIDDLE EAST FORCES AND EGYPT

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THE value of the Weil-Felix test in the diagnosis of the typhus group of exanthemata is so firmly established that we can add little to the extensive literature on this subject. Information received from workers situated in different countries where typhus-like diseases are endemic and epidemic indicates that a measure of correlation exists between the clinical variety of the disease and the strain of *B. proteus* X19 agglutinated. The position has been fully summarised by Felix (1933), Harvey (1933), and numerous others, and may be epitomised as follows :—

In classical Old or New world typhus fever *B. proteus* X19 is strongly agglutinated, OX₂ slightly, and OXK unaffected. Similar reactions have been recorded in Mexican Tabardilla; South-Eastern United States Endemic typhus or Brill's disease; Toulon Naval Fièvre nautique; Australian Endemic typhus, and Malayan and Dutch East Indies Tropical W, or Shop typhus. Also to be included in this category is Brazilian São Paulo endemic typhus, in which slight OXK agglutination is caused. Results opposite to the above are obtained in Tropical K type Scrub typhus as well as Japanese, Malayan and Sumatran Tsutsugamushi mite-borne fever where *B. proteus* OXK alone is actively clumped.

Occupying an intermediate position are Eastern and Western United States Rocky Mountain Spotted Fever, Italian Febbre Eruttiva, and South African Tick Bite Fever, in all of which OX₁₉, OX₂, and OXK are affected to a comparatively low titre, and very similar is the response elicited in Marseilles and Tunis Fièvre Boutonneuse with the exception that agglutinins for OXK are absent. Probably Indian Tick typhus also belongs to the latter class, but its position is by no means clear,

as Boyd (1935) has shown. In a study of fevers of the typhus group in India he reported 14 cases wherein agglutination was predominantly of the OX₂ type. But Boyd remarked that "in view of the results obtained by Felix (1933), in testing sera of cases of *Fièvre Boutonneuse*, where some gave a titre of 2000 for X₁₉ and nil for X₂, whilst others gave a titre of 2000 for X₂ and nil for X₁₉, it seems both reasonable and probable that the above group do not have X₂ as the *main* antigen of their virus but as a group reaction to some other virus." Continuing, Boyd stated: "Although therefore there is much in favour of the hypothesis that three types of typhus fever as suggested alone exist, it is by no means impossible that X₂-X₁₉ cases have one unknown main antigen in common, and that the varying X₂ and X₁₉ titres are either individual idiosyncrasies in a group reaction, or are related to passage of the virus through different vectors." Thus, claims for the establishment for an independent clinical entity of typhus based on predominant OX₂ agglutination rest on a precarious foundation. Also noteworthy is the fact that in Australian Q and Montana fever, two recently described rickettsial diseases, agglutinins for *B. proteus* are not developed (Dyer, 1939, and Freeman *et al.*, 1940).

Wolbach (1940), in his authoritative article on this subject, has epitomised the inter-relationship of the three principle strains of *B. proteus* and the three main groups of human typhus thus:—

Typhus Group.	Tsutsugamushi Group.	Spotted Fever Group.
<i>B. proteus</i> OX ₁₉ +++	<i>B. proteus</i> OX ₁₉ —	<i>B. proteus</i> OX ₁₉ +
<i>B. proteus</i> OX ₂ +	<i>B. proteus</i> OX ₂ —	<i>B. proteus</i> OX ₂ +
<i>B. proteus</i> OXK—	<i>B. proteus</i> OXK+++	<i>B. proteus</i> OXK+

+++ High titre agglutination; + low titre clumping; — negative.

From the above it will be appreciated that the Weil-Felix test is an undoubted aid to the clinical recognition of at least two of the more important varieties of typhus fever. From the scientific point of view, however, the position is unsound, because the Weil-Felix test is a heterogenetic antigen-antibody reaction the specificity of which was accidentally discovered as the outcome of a fortuitous event. No satisfactory explanation for its occurrence has as yet been forthcoming, and the belief that the rickettsiae of typhus represent mutants of *B. proteus* is no longer tenable (Wolbach, 1940). Recently, light has been shed on this problem by the work of Zinsser (1937), Castaneda and Zia (1933), and Castaneda (1935), who discovered the existence of an antigenic fraction, presumably of the carbohydrate class, common to both *B. proteus* OX₁₉ and murine and epidemic rickettsiae.

Current work on the rickettsial infections has demonstrated that they constitute a diverse group of micro-organisms, disseminated by different vectors, differing in their degree of pathogenicity to man and animals, and possessing widely different immunological and biological

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characteristics. Likewise *Bacillus proteus* is a bacterium widely distributed in nature and antigenically not of a homogeneous species. If therefore the future scope of the Weil-Felix reaction were to develop along logical lines, it would be necessary to discover which strains of *B. proteus* were most closely related antigenically to individual rickettsiae and subsequently to employ such strains for diagnosis of the associated clinical conditions.

It has already been proposed by Felix (1933) that if local strains of *B. proteus* were to be obtained from cases of Rocky Mountain Spotted Fever, Fièvre Boutonneuse, African Tick Fever, and Indian Tick Fever, they might assist towards their serological diagnosis. But in the same publication it was admitted by Felix that although such a strain of *B. proteus* was isolated by Dr Lima of São Paulo from a child who had recovered from an attack of São Paulo typhus and designated *B. proteus* XL, unfortunately, sera of persons convalescent from São Paulo typhus agglutinated *B. proteus* X19 to higher titre than the patient's own XL organism. Moreover, Felix found that immunisation of rabbits with strain XL resulted in the formation of group agglutinins for OX2 and OXK, whereas a pure OX19 strain did not behave similarly. The problem therefore is a complex one, involving a multiplicity of factors, and whereas a positive Weil-Felix result is unequivocal proof of certain forms of typhus, a negative or only weakly positive reaction may be elicited in the remainder. Thus the full significance of OX2 agglutination remains unexplained (see Boyd, 1935, Mason and Alexander, 1939, and Alexander, Mason and Neitz, 1939).

Another aspect of the Weil-Felix test worth considering is that of false positive reactions. For example, in Undulant Fever, *B. proteus* suspensions may be clumped to a serum end-titre of 1 : 240 (see Kemp, Wright and Wayne, 1933), and in chronic Brucellosis *B. proteus* may be agglutinated to a higher titre than the homologous infecting bacterium (see Foshay, 1940 and Calder, 1941). The presence of heterophile agglutinins such as these does not present a serious obstacle to diagnosis, because they remain static throughout the course of the illness and disappear promptly during convalescence. Moreover, by means of cross-absorption tests with suspensions of *B. proteus* and *Br. melitensis*, the specificity of anti-brucella agglutinins can be demonstrated in undulant fever (see Findlay, 1941). Within recent years a better laboratory test for the diagnosis of typhus has been evolved, and workers in America and Australia have directed their attention to the study of human specific rickettsial agglutinins.

Among pioneer investigators were Otto and Dietrich (1917) and Weigl (1923), who inoculated lice intra-rectally with infective material and so prepared from the gut of the louse a suspension of rickettsiae sufficient for a few agglutination tests (see also Mariani, 1940). The laborious task of obtaining rickettsial suspensions by such a technique restricted the practical application of the tests, and the matter received

scant attention until Zinsser and Castaneda (1932a) produced relatively pure suspensions on rickettsiæ in the peritoneum of X-rayed rats.

Subsequently, Zinsser and Castaneda (1932) employed such antigen and demonstrated the serological individuality of the Mexican murine and European louse-borne varieties of the disease (see Zinsser, 1940, for summary of results). Another method of procuring suspension of murine *rickettsiæ* suitable for macroscopical agglutination has been employed by Castaneda (1939) and Hudson (1940), who utilised as a source of material the lungs of infected rats and mice. The latter's results showed specific agglutination between antigen and patient's sera up to dilutions varying from 1/320 to 1/128,000. Durand and Giroud (1940) cultivated *R. prowazeki* in guinea-pig and rabbit lungs, prepared suspensions of organisms, by differential centrifugalisation and demonstrated the development of agglutinins in persons immunised with vaccine of the same origin. Two important discoveries which have enhanced knowledge of this subject have been the work of Zia (1934), who grew the rickettsia of epidemic and murine typhus in the chorio-allantoic membrane of the developing chicken embryo; secondly that of Cox (1938), who found that these organisms multiplied freely in the yolk-sac of the fertile hen's egg. Later, Cox and Bell (1939), studying strains of *R. diaporica* recovered from *Dermacentor andersoni* the vector of Montana rocky mountain spotted fever, successfully grew the rickettsiæ in eggs, prepared an antigen, and showed the presence of agglutinins in patients' sera (see also Davis, 1939). Likewise, Burnet and Freeman (1939), working with Queensland Q fever disease epizootic in the Queensland bandicott *Isodontorossus* Ramsay (usually propagated among rats and occasionally communicated to man by the tick *Hæmaphysalis humerosa*), cultivated *R. burneti* in eggs and obtained suspensions for agglutination tests. Burnet and Freeman studied the antigenic behaviour of *R. diaporica* and *R. burneti*, and by the use of cross-immunological agglutination tests established the similarity of the two diseases. Dyer (1939) observed that *R. burneti* was agglutinated to higher titre by the serum of a laboratory worker who had been accidentally infected with the Montana disease. Yet another illustration of studies conducted on similar lines has been the recent work of Bengston (1941), who employed agglutination and agglutinin-absorption tests to demonstrate the close immunological relationship of the rickettsiæ of Australian Q to American X fever (see also Cox, 1940).

With the help of rickettsial agglutination reactions Burnet and Freeman's (1939) observations were extended by Freeman *et al.* (1940), who studied the natural distribution of Q fever infection among Australian wild animals. It is worth reiterating that in both diseases strains of *B. proteus* X are not affected (Dyer, 1939, and Burnet and Freeman, 1939), and so it is that our knowledge of their taxonomical position, nomenclatural status, respective ætiological agents has been culled from a study of rickettsial agglutination results, and not from Weil-Felix

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tests or animal inoculation effects. The above researches on Montana and Q fever illustrate the increasing practical utility of the rickettsial agglutination reactions and synchronously the increasing limitations of the Weil-Felix test in the diagnosis of the group of typhus fevers. Recently Bengston (1941a) and Bengston and Topping (1941), using rickettsial antigen, were able to show the presence of specific complement fixing antibodies in the sera of patients suffering from endemic typhus, rocky mountain spotted fever and Q fever. Complement fixation tests thus promise to be an additional serological method for the diagnosis of human typhus, and a valuable enhancement to our knowledge of the subject.

Authors' Work.—We have performed a series of Weil-Felix and rickettsial agglutination tests on sera of typhus cases affecting British and Allied troops stationed in Egypt, Palestine, Syria and Iraq, and we have also had free access to civilian sources of clinical material.

Antigens Employed.—Standardised Royal Army Medical College suspensions of *B. proteus* OX19, OX2 and OXK have been employed uniformly. Two rickettsial suspensions have been used, prepared by Professor James Craigie of Toronto University. The epidemic strain was isolated by Dr Briehl in Central Europe, and the murine strain by Professor Zinsser in Mexico. Both varieties have been continuously propagated in the yolk-sac of the developing chicken embryo.

Technique of the Weil-Felix and Rickettsial Agglutination Reaction Tests.—Successive dilutions of patients' serum ranging from 1/100 to 1/1600 and over when necessary were made in 0.86 per cent. saline solution in Dreyer's tubes. To 0.4 c.c. of each serum dilution was added 0.4 c.c. of a 1/15 dilution of *B. proteus* concentrated suspensions made up in 1/20 Sorensen's ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) sodium potassium phosphate buffer of pH 7.2 and each serum was put up in triplicate against *B. proteus* OX19, OX2 and OXK. In the case of rickettsial antigen concentrated material was diluted 1/20 in buffered phosphate saline solution of pH 7.2, and an equal bulk added to each serum dilution. Mixtures were incubated for 4 hours at 42° C., removed, placed overnight in the ice-chest, and the results read in the morning. By holding the tubes under a strong light with a dark background, results were easily seen in the case of the Weil-Felix test. With the rickettsial agglutination reaction care is essential, since clumping is exceedingly fine in character and is liable to escape detection. We have found that

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the best method is to hold the tube up to the light and then with forefinger and thumb sharply rotate the tube twice. If agglutination is present it will be visible as a fine granular flocculum which arises into the supernatant fluid. Should difficulty be experienced in the reading of the test, the supernatant clear fluid should be discarded, a film made from the deposit of agglutinated rickettsiæ stained by Macchiavello's method, and examined under the microscope for signs of clumping. Obviously both the saline and normal serum controls must be treated likewise for purpose of comparison and should remain opalescent and free from deposit.

Observations on Civilian and Military Cases.—The civilian authorities have supplied us with information regarding the seasonal incidence of typhus in Egypt and in Palestine. In Egypt the greatest number of cases occur between February and June and the death-rate is about 13 per cent. In Palestine the maximum incidence is from August to November and the

TABLE I

Typhus among Egyptians. Summary of Clinical and Laboratory Results

Case No.	Locality.	Clinical and other Data.	<i>B. proteus</i> .			Rickettsiæ.		Diagnosis.
			OX19.	OX2.	OXK.	Epidemic.	Murine.	
E. 1	Cairo	Typical. Convalescent	1/1280	Neg.	1/40	1/2560	1/320	Epidemic
E. 2	Cairo	Typical. Convalescent	1/640	1/80	Neg.	1/2560	1/640	Epidemic
E. 3	Cairo	Typical. Convalescent	1/320	1/100	Neg.	1/1280	1/320	Epidemic
E. 4	Cairo	Typical. Convalescent	1/800	Neg.	1/40	1/2560	1/160	Epidemic
E. 5	Cairo	Typical. Convalescent	1/1280	1/320	Neg.	1/1280	1/160	Epidemic
E. 6	Cairo	Typical. Convalescent	1/1600	1/400	1/800	1/400	1/1600	Murine
E. 7	Cairo	Typical. Fatal. Blood taken at P.M.	1/800	1/400	1/1600	1/800	1/100	Epidemic
E. 8	Cairo	Typical. 3 days convalescent	1/1600	1/800	1/200	1/800	1/3200	Murine
E. 9	Cairo	Typical. 8 days convalescent	1/400	1/200	1/200	1/500	1/200	Epidemic
E. 10	Cairo	Typical. 6 days convalescent	1/4000	1/400	1/400	1/1600	1/200	Epidemic
E. 11	Cairo	Typical. 3 days convalescent	1/400	1/100	1/3200	1/200	1/400	Murine
E. 12	Tanta	Severe case. Convalescent	1/1600	1/200	1/800	1/6400	1/800	Epidemic
E. 13	Tanta	Typical. Convalescent	1/10,000	1/800	1/400	1/400	1/2000	Murine
E. 14	Tanta	Convalescent	1/1600	1/50	1/100	1/800	1/400	Epidemic
E. 15	Tanta	Typical. Convalescent 7 days	1/5000	1/200	1/200	1/500	1/400	Epidemic
E. 16	Tanta	Typhus. 1 day convalescent. Pregnant female	1/5000	1/100	1/200	1/1200	1/400	Epidemic
E. 17	Tanta	Typhus fever. 8 days. Pregnant female. GP+	1/300	...	...	1/200	1/50	Epidemic
E. 18	Tanta	Typhus. 12th day. GP—	1/400	1/200	1/300	1/3200	1/800	Epidemic
E. 19	Tanta	Hæmorrhagic typhus. 8th day moribund. GP+	1/400	1/3200	1/800	1/300	1/150	(?) Epidemic.
E. 20	Tanta	Typhus. 7 days duration. GP+	1/400	...	...	1/400	Neg.	OX2 type Epidemic
E. 21	Tanta	7th day of illness. Typical. GP—	1/100	1/200	1/200	1/200	1/200	(?)
E. 22	Tanta	18th day of illness. 4 days convalescent. Complicated by parotitis	1/400	1/800	1/400	1/3200	1/600	Epidemic
E. 23	Tanta	10th day of typical fever	1/1600	1/100	1/300	1/1200	1/200	Epidemic

Guinea-pig inoculation positive, GP+.
Guinea-pig inoculation negative, GP—.

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death-rate is very low. It would appear that the murine flea-borne typhus of Palestine differs considerably from and is much milder than the epidemic louse-borne variety prevalent in Egypt.

Through the kindness of the directors of the Cairo Fever Hospital and the fever hospital at Tanta, we have been able to observe large numbers of cases of typhus at different stages of the illness. A summary of the laboratory data in certain of these cases is given in Table I. From six of the Tanta cases blood was removed, and at the bedside two guinea-pigs were inoculated with 5 c.c. Four of these tests were positive. Our findings support the view that the local disease in Egypt is epidemic in type.

We have also examined sera submitted from 50 soldiers suffering from typhus and treated in various hospitals in Egypt, the Suez Canal area, Palestine, Syria, Iraq, Iran and Eritrea. Full case notes were available, and the diagnosis in all cases may be regarded as established beyond doubt. The laboratory findings are shown in Table II.

It would appear that the disease in soldiers is identical with that observed among civilians. It is likely that the low death-rate recorded is directly attributable to the good nutritional state of the soldiers and to the skilled nursing which they receive. Certain units, such as the R.A.S.C. and the R.A.O.C., are more exposed to attack than others; the greater the contact with native labour the greater the number of cases notified. There is not the slightest evidence that there has been any case-to-case spread among the soldiers; civilian sources of infection remain the real danger. The small series of cases reported is in itself an amazing testimony to the very effective hygienic facilities available to the Services.

Control Experiments. Specificity of the R.A.R. Test.—Seldom does normal human serum contain agglutinins for *B. proteus* strains OX19, OX2 and OXK in dilutions greater than 1/100. This has been repeatedly observed by many investigators throughout the world, and recently Buchwald (1941) arrived at this conclusion after he had studied the distribution of natural agglutinins for *B. proteus* strains in 300 normal sera. Precisely similar deductions were arrived at by myself (C. E. v. R.) in 1940 in Scotland after having conducted a similar survey of the sera of 176 healthy Polish soldiers and a like number of Scotsmen. Approximately 8 per cent. of the samples tested showed slight agglutination of one or other strain of *B. proteus*, in dilutions ranging from 1/20 to 1/80, and about 1 per cent. agglutinated X19 up to a 1/120 titre. Such agglutinins appeared

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to be thermostable in character and resisted heating at 56° C. for thirty minutes. Likewise, sera exhibiting low-titre reactions did not appear to be connected with any ascertainable factor

TABLE II
Typhus among British and Allied Troops in the M.E.F.

Case No.	Locality.	<i>B. proteus</i> .			Rickettsiæ.		Diagnosis.	Day of Illness.
		OX19.	OX2.	OXK.	E.R.	M.R.		
1	Iraq	1/1600	1/1600	1/80	1/1600	1/200	Epidemic (OX2 type)	35
2	Iraq	1/1600	1/100	Neg.	1/320	1/80	Epidemic	43
3	Iraq	1/1600	1/80	Neg.	1/320	1/80	Epidemic	28
4	Iraq	1/1600	1/200	1/200	1/300	1/800	Murine	42
5	Iraq	1/1600	1/800	1/800	1/800	1/400	Epidemic	42
6	Iraq	1/1600	1/1600	1/1600	1/1200	1/400	Epidemic (OX2 type)	41
7	Iraq	1/1600	1/400	1/400	1/1600	1/400	Epidemic	40
8	Beirut	1/1600	1/200	1/400	1/80	1/400	Murine	24
9	Tel-el-Kebir	1/1600	1/200	1/1600	1/400	1/2000	Murine	26
10	Alexandria	1/3200	1/100	1/200	1/1600	1/400	Epidemic	8
11	Beirut	1/400	1/100	1/200	1/200	1/2000	Murine	...
12	Kantara	1/250	1/500	1/50	1/400	1/3200	Murine	26
13	Alexandria	1/3200	1/400	1/200	1/600	1/400	Epidemic	18
14	Mersa Matruh	1/600	1/800	1/100	1/400	1/800	Murine	11
15	Cairo	1/1600	1/100	Neg.	1/1600	1/800	Epidemic	13
16	Haifa	1/3200	1/100	1/100	1/800	1/1600	Murine	16
17	Alexandria	1/3200	1/400	1/400	1/3200	1/400	Epidemic	...
18	Gaza	1/5000	1/800	1/800	1/200	1/1600	Murine	19
19	Kanatar	1/400	1/200	1/400	1/200	1/400	Murine	10
20	Cairo	1/1600	1/100	1/200	1/200	1/400	Murine	...
21	Kantara	1/1600	Neg.	1/300	1/1200	1/200	Epidemic	14
22	Sarafand	1/400	1/5000	1/400	1/200	1/300	Murine (OX2 type)	18
23	Helwan	1/400	1/1000	1/1000	1/2000	1/1000	Epidemic (OX2 type)	11
24	Mersa Matruh	1/800	1/200	1/800	1/200	1/400	Murine	10
25	Kantara	1/5000	1/400	1/300	1/200	1/2000	Murine	27
26	Cairo	1/1600	1/100	1/200	1/200	1/2000	Murine	23
27	Cairo	Nil	1/100	1/200	1/200	1/400	Murine	43
28	Cairo	1/800	1/1000	1/1200	1/200	1/400	Murine (OX2 type)	19
29	Tel-el-Kebir	1/1600	1/200	1/200	1/1600	1/2000	Murine	...
30	Geneifa	1/2000	1/400	1/150	1/150	1/3200	Murine	12
31	Geneifa	1/600	1/3200	1/500	1/200	1/50	Epidemic (OX2 type)	10
32	Asmara	1/4000	1/400	1/500	1/500	1/5000	Murine	27
33	Beirut	1/8000	1/800	1/400	1/200	1/3200	Murine	15
34	Beirut	1/2000	1/250	1/1000	1/200	1/3200	Murine	43
35	Beirut	1/500	1/150	Neg.	1/100	1/1200	Murine	64
36	Gaza	1/100	1/3200	1/200	Neg.	1/50	Murine (OX2 type)	25
37	Geneifa	1/200	1/3200	1/800	1/100	1/400	Murine (OX2 type)	28
38	Geneifa	1/400	1/400	1/800	1/2000	1/5000	Murine	15
39	Geneifa	1/5000	1/150	1/400	1/2000	1/6400	Murine	15
40	Geneifa	1/50	1/800	1/200	1/200	1/1600	Murine	11
41	Geneifa	1/6400	1/200	1/150	1/800	1/6400	Murine	16
42	Cairo	1/1000	1/40	1/125	1/800	1/400	Epidemic	11
43	Cairo	1/2000	1/1600	1/800	1/3200	1/1000	Epidemic (OX2 type)	30
44	Kantara	1/400	Neg.	1/25	1/200	1/400	Murine	9
45	Kantara	1/1200	Neg.	1/50	1/400	1/1600	Murine	11
46	Jerusalem	1/800	Neg.	Neg.	1/50	1/3200	Murine	33
47	Beirut	1/5000	1/150	1/200	1/400	1/1600	Murine	12
48	Alexandria	1/1600	1/200	1/150	1/200	1/1200	Murine	...
49	Cairo	1/400	Neg.	1/200	1/800	1/100	Epidemic	9
50	Port Said	1/350	1/50	1/50	1/250	1/2000	Murine	25
51	Port Said	1/500	1/800	1/400	1/400	1/6400	Murine	24
52	Geneifa	1/2000	1/800	1/400	1/250	1/5000	Murine	13
53	Geneifa	1/2000	1/100	1/100	1/250	1/5000	Murine	13
54	Sarafand	1/5000	1/400	1/400	1/400	1/12800	Murine	20
55	Geneifa	1/2000	1/100	1/100	1/400	1/1600	Murine	11
56	El Ballah	1/2500	1/400	1/100	1/200	1/1600	Murine	...

and were met with indiscriminately. Such non-specific agglutinins were unrelated to the Wassermann reaction, and were absent in the sera of 10 Poles who had suffered from typhus exanthematicus

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or been actively immunised with Weigl's vaccine two or three years previously. (See also Liu and Zia, 1940.) On the other hand, a number of positive readings were returned from Scotsmen who had never left their native heath. Results such as the above offer conclusive evidence of the non-specific character of low-titre *B. proteus* agglutination; consequently any positive Weil-Felix test result of under 1/100 dilution must be ignored and regarded as normal.

But in typhus fever the most striking feature of the Weil-Felix test is the singular regularity with which high-titre positive results occur, and moreover such agglutinins are thermostable at 55° C. for thirty minutes. The same applies to true specific anti-rickettsial agglutinins, and from a series of preliminary experiments we were satisfied that heating to 55° C. for half-an-hour did not annul the agglutinating properties of sera derived from either epidemic or murine varieties of infection.

Results with Normal Sera.—One hundred normal sera from healthy blood donors have been tested in quintuplicate against suspensions of OX19, OXK, OX2, E.R. and M.R. Once again the results showed that non-specific agglutinins for strains of *B. proteus* were not uncommon in low-titre ranging from 1/25 to 1/150. We had also reason to suspect that every batch of *B. proteus* suspensions was not endowed with the same degree of physical stability, and different lots of antigen did not always give identical results, in particular *B. proteus* OXK.

Another point worth mentioning is that in the case of low-titre (<1/100) non-specific agglutination the results were often difficult to interpret, and clumping when it occurred was incomplete and often hard to distinguish from sedimentation. However, difficulties accruing from this source were not insurmountable, because in typhus fever agglutination is invariably of the large flake coarse variety, attended by a heavy flocculum and clearing of the supernatant fluid, and often present in high-titre. For practical purposes of typhus diagnosis any positive reading under 1/100 serum dilution may be ignored, but for academic interest we have examined the behaviour of sera exhibiting low-titre *B. proteus* agglutination to discover the reaction of these sera against suspensions of rickettsiæ in similar low dilution. Thus, with sera which gave weakly positive OX19, 2 or K agglutination, a comparison was made between this effect and the action of the same sera against suspensions of epidemic and murine rickettsiæ. The results were interesting,

and showed that out of a total of 100 normal sera examined 22 yielded low-titre Weil-Felix reactions, against 1 which showed clumping of epidemic rickettsiæ up to 1/50. It thus appears that suspensions of rickettsiæ are less liable to show non-specific low-titre agglutination than does *B. proteus* antigen. Three explanations are conceivable. It may be that low-titre agglutination of *B. proteus* observed in normal and pathological sera (other than from cases of typhus: see table) are in reality true *B. proteus* agglutinins, or else normal sera lack anti-rickettsial agglutinins. Alternatively, it is possible, since the suspensions of rickettsiæ employed are finely dispersed and physically more stable, that suspensions of *B. proteus* agglutination in low dilution may be difficult to detect, and so convey a false impression of the apparent degree of specificity of the reaction.

Control Tests on Pathological Sera.—The following series of experiments were performed on 74 samples of blood removed from 12 cases of typhoid fever: 1 case of paratyphoid A infection; 20 cases of benign tertian malaria; 5 cases of malignant tertian malaria; 4 cases of relapsing fever which had been acquired in the Western Desert; and 10 cases of acute polio-encephalitis. Also included were 22 cases of sandfly fever contracted by a unit serving in the desert at the height of the sandfly season. Table III contains a summary of the results. It shows very clearly

TABLE III
Control Experiments

Serum Examined.	No. of Cases Tested.	OX19.		OX2.		OXK.		E.R.		M.R.		Total Sera showing no W.F. Reactions (in 1/100 and over) and R.A.R. Reactions (in 1/50 or over)
		Positive Sera of 1/100 Dilutions and over.	Highest Titre Recorded.	Positive Sera of 1/100 Dilutions and over.	Highest Titre Recorded.	Positive Sera of 1/100 Dilutions and over.	Highest Titre Recorded.	Positive Sera of 1/50 Dilutions and over.	Highest Titre Recorded.	Positive Sera of 1/50 Dilutions and over.	Highest Titre Recorded.	
Normal	100	1	1/200	11	1/200	14	1/200	1	1/50	1	1/50	78
Malaria (BT)	20	1	1/100	1	1/200	5	1/100	1	1/100	2	1/100	11
Malaria (MT.)	5	Nil	...	Nil	...	1	1/100	Nil	...	Nil	...	5
Sandfly fever	22	2	1/150	4	1/200	10	1/200	Nil	...	1	1/50	10
Typhoid fever	12	Nil	...	Nil	...	1	1/100	1	1/50	1	1/50	10
Convalescent												
Paratyphoid A	1	Nil	...	Nil	...	Nil	...	Nil	...	Nil	...	1
Spirochaetal Relapsing fever	4	Nil	...	Nil	...	2	1/100	Nil	...	Nil	...	2
Acute polio-encephalitis	10	1	1/100	1	1/100	3	1/100	Nil	...	1	1/50	5
Typhus	73	73	1/16,000	58	1/3200	57	1/3200	See Tables I and II	1/6400	See Tables I and II	1/12800	Nil

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that in certain acute febrile diseases a positive Weil-Felix test of low-titre may be expected. Particularly in respect of sandfly fever where a substantial proportion of specimens agglutinated *B. proteus* OXK. In contrast to these findings, the rickettsial agglutination results afford an interesting comparison and illustrate the high degree of specificity of such agglutination for typhus fever.

Discussion

The W.F. and R.A.R. Tests Compared.—It may be said with conviction that the Weil-Felix and rickettsial agglutination reaction tests show close agreement in their presence at various stages of typhus. Castaneda, Silva and Monnier (1940) contend that agglutinins for *R. prowazeki* can be detected on the sixth day of illness and appear earlier than agglutinins for *B. proteus* X19. We ourselves have not confirmed this point, but can state that both increase in titre throughout the second week of illness, attain a maximum about the commencement of the third week, and thereafter decline. Our work indicates that heterophile agglutinins for *B. proteus* disappear more quickly than specific anti-rickettsial agglutinins. This may be due to the fact that anti-rickettsial agglutinins occur in higher titre than do agglutinins for *B. proteus*, as already found by Hudson (1940). The latter reported positive clumping in serum dilutions varying from 1/320 to 1/128,000, and we can confirm his findings in this respect.

Inter-relationship of E.R. and M.R. Agglutination.—Zinsser and Castaneda (1932), working with Weigl's European infected louse gut vaccine and murine rickettsiae cultivated in the peritoneum of X-rayed rats, discovered that the serological association of the two organisms resembled the relationship of *B. paratyphosus* *B.* of different origins. Rabbits immunised with three doses of Weigl's vaccine showed a homologous agglutinin titre of 1 : 500 and one of 1 : 100 against the Mexican murine strain. Likewise, if rabbits received parenteral doses of murine vaccine, the homologous antigen was agglutinated to an end titre of 1 : 1000 and the epidemic Weigl strain rickettsial to a 1 : 100 dilution. By the use of rickettsial agglutination tests Zinsser and Castaneda (1932, 1932*a*, 1933, 1934) have successfully demonstrated the serological individuality of European louse-borne epidemic and murine flea-borne Mexican typhus, and our experience in the Middle East has provided ample confirmation of their researches. We have shown the correlation

which exists between the clinical typhus fever, *B. proteus* and *R. prowazeki* agglutinins: Secondly, the antigenic relationship of epidemic and murine rickettsiæ. For instance, we have frequently noticed that in cases of typical louse-borne epidemic typhus the M.R. agglutination titre is roughly one-third or less of the E.R. result. Zinsser and Castaneda's (1932) figures likewise suggest values of the same magnitude. We also agree with these workers that in murine typhus the N.R. to E.R. serum titre ratio is considerably greater, *e.g.* seven times or more.

Incidence of Epidemic and Murine Typhus.—The second aspect of our studies has automatically raised the question as to the relative distribution of murine and epidemic typhus in the Middle East area. At least a thousand cases drawn from and representative of a wide territorial area over a period of years would be required before a satisfactory reply can be given. Meanwhile we have tested sera from 73 cases of typhus occurring among military and civilian subjects over a period of eight months, and certain definite conclusions can be drawn from our preliminary observations. The results show that for the months March, April, May and June, when the incidence of typhus was at its maximum for the year 1942, of a total of 23 civilian sera examined 20 were epidemic and 3 of the murine type. For approximately the same period 23 sera from military personnel in Egypt were tested, and of these 7 were epidemic and 16 murine. Epidemic louse-borne typhus thus seems to be the predominant type of disease among civilians and murine type in soldiers for the period specified. In present times of swift transport and mass movements it would be a bold venture to try to subdivide the type incidence of the disease according to geographical localities, but nevertheless we have noticed that whereas the majority of the cases occurring in the Cairo, Alexandria and Tanta areas were of the epidemic type, the greater proportion derived from the Suez Canal zone were murine typhus. In consequence, one is sorely tempted to speculate on the probability of the city disease being louse-borne and the Canal area cases being disseminated by fleas, presumably associated with the presence of shipping and rats. Seven sera received from Palestine and Syria have all been of the murine type. This is in accordance with what is already well known, namely, that epidemic typhus rarely occurs in this part of the Middle East. Figures for typhus in Syria are unobtainable, but Professor Dennis of Beirut has informed me that the situation is similar to that in Palestine and

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that the disease is of a comparatively mild type in both countries and of low mortality (see Reitler *et al.* 1939). We would like to refer to a group of 10 clinically typical typhus cases in which *B. proteus* OX2 agglutination has been predominant, the titre reading 1/1000 or more.

Five of the cases showing simultaneous high-titre clumping of OX19 and E.R. are suggestive of the existence of a common antigen to the epidemic typhus group. Four other cases gave a high-titre OX2 agglutination without much OX19, K, E.R. or M.R. reaction; and one was vaguely suggestive of tick typhus. In two of these patients the disease was severe enough to cause death, but the remainder did not seem to possess any distinguishing clinical characteristics. An explanation of these findings is hard to seek, and further effort must be made to elucidate the nature of apparently specific OX2 type agglutination. Pijper and Crocker (1938) maintained that in South African epidemic typhus *B. proteus* OX19, OX2 and OXK are all agglutinated in low dilution, whereas in European epidemic typhus OX19 alone is agglutinated. Gear (1938), on the contrary, has obtained different results.

There has been no evidence to hint that our cases in the M.E.F. were associated with the bites of ticks or that relapsing fever occurred in these districts simultaneously. In general, rickettsial agglutination tests should prove invaluable in elucidating the precise nature of all suspected cases of typhus, and may be regarded as a valuable laboratory aid to the differential diagnosis of epidemic and murine typhus. Their introduction holds much promise for the future, and with the use of antigens prepared from locally isolated strains of rickettsiæ further advances may be anticipated. Before ending this discussion we would like to draw attention to the rigorous control experiments which have been performed in order to prove the practical value of the R.A.R. as a diagnostic procedure. One hundred and seventy-four control tests have been done, as against seventy-three tests for typhus.

The results obtained have offered conclusive proof of the remarkable physical stability and serological specificity of the rickettsia suspensions prepared by Dr James Craigie of Toronto University, to whom we owe our many thanks.

Conclusions

(1) Fifty military and 23 civilian cases of typhus fever from the Middle East area have been tested for serological evidence of agglutinins against *B. proteus* OX19, OX2 and OXK, as well as agglutination of epidemic and murine rickettsiæ.

(2) Results show close correlation between the Weil-Felix (W.F.) and rickettsial agglutination (R.A.R.) tests.

(3) Control experiments to verify the specificity of the R.A.R. have been performed on 100 normal human sera. Also, 74 pathological sera derived from 25 cases of malaria, 12 of typhoid, 22 of sandfly fever, 4 of spirochætal relapsing fever, and other febrile conditions, all of which were carefully selected and accurately diagnosed. Results have shown that the R.A.R. test did not give false positive agglutination in the diseases specified.

(4) The R.A.R. is a highly specific and reliable test for typhus fever which is equal if not superior to the W.F. test. It possesses the advantage of differentiating between the epidemic and murine varieties of infection, which the W.F. reaction cannot do. The principle technical disadvantage of the R.A.R. is that rickettsial agglutination is very fine in character and liable to be missed, whereas agglutination of *B. proteus* is easily visible.

(5) Both reactions become positive from the seventh day of illness, *i.e.* usually two days after the rash has appeared. The agglutinin titre reaches its maximum about the fourteenth day of illness and thereafter declines at different rates. *B. proteus* agglutinins recede more quickly than those for rickettsiæ. In a case of epidemic typhus the R.A.R. was still present in high titre on the sixty-fourth day after onset of symptoms when the W.F. test was fast disappearing.

(6) In severe epidemic Egyptian typhus, showing high-titre W.F. test reactions, epidemic rickettsiæ are usually agglutinated to a similar or greater serum dilution. Simultaneously, low-titre cross-agglutination for murine rickettsia suspensions occurs, and the E.R./M.R. ratio is approximately in the order of 3 : 1.

(7) In both Palestinian and Syrian mild murine typhus, which yield high-titre OX19 agglutination, the homologous rickettsiæ are strongly clumped, frequently to even higher titre with some cross agglutination of epidemic rickettsiæ in lower dilution, the M.R./E.R. ratio being roughly 10 : 1.

(8) Results indicate that during winter months in Egypt louse-borne epidemic typhus is the prevalent disease among Egyptians living in the Cairo and Tanta areas.

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(9) There has been remarkably little typhus fever among British and Allied troops of the Middle East Force during 1942. From a study of 50 cases it would seem that even during the winter, when the incidence of epidemic typhus was at its maximum among the civilians of Cairo and Tanta, many of the Army cases were murine infections. It also seemed that the murine disease was more prevalent in the Suez Canal area than in the cities. So far, in 1942, in Palestine and Syria the disease has remained exclusively murine in type.

(10) The occurrence of cases of typhus fever (presumably not due to tick bite) has been observed by us in Egypt and Palestine in which OX₂ agglutination predominates without marked E.R. or M.R. agglutination.

(11) All sera derived from suspected cases of typhus should be tested in quintuplicate for presence of agglutinins for *B. proteus* OX₁₉, OX₂, OXK, epidemic and murine rickettsiae.

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