THE REPUTED ANTIGENIC RELATIONSHIP BETWEEN ORGANISMS OF THE BRUCELLA GROUP ON THE ONE HAND, AND OF THE PASTEURELLA, PFEIFFERELLA AND PROTEUS GROUPS ON THE OTHER

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DURING the past few years certain statements have appeared in the literature suggesting that the agglutination reaction, which is generally considered to afford evidence of infection with a specific micro-organism, is in fact often liable to misinterpretation as the result of infection of the host with generically different but antigenically related organisms. The evidence on which this contention has been based has consisted largely in the demonstration in a given serum of agglutinins for two or more entirely different bacteria.

The authors of these papers do not seem to have been aware of the frequent existence of so-called "normal" agglutinins in human and animal sera, nor to have taken account of the occurrence of agglutinins in serum due to a latent or past infection. That numerous instances occur of the possession of a common antigen by specifically distinct organisms within the same genus, and that a few instances are known of the sharing of such an antigen by two generically dissimilar organisms, for example *Pneumococcus* Type II and *Bact. friedländeri* Type B, is not questioned for a moment, since they are supported by adequate immunological evidence. The main point at issue is to determine whether the mere co-existence in a given serum of agglutinins to two different organisms can be accepted as sufficient evidence of antigenic affinity between them. The interpretation to be put on the finding of such a co-existence must depend on the type of serum examined; whether, for instance, it is the serum of an apparently normal or of a diseased animal, or whether it is the serum of an animal that has been artificially immunised against a particular micro-organism. In the latter case it is important to know whether antibodies to either organism were present before the commencement of immunisation.

RECORDS OF PREVIOUS WORKERS

Mallmann (1930) observed that a cow serum, which agglutinated *Br. abortus*, likewise agglutinated *Pasteurella bovisepitica*, *Past. aviseptica*, and *Pfeifferella mallei*. Cross-agglutination studies conducted with the sera of rabbits experimentally immunised with *Brucella* and *Pasteurella* strains showed a high degree of interagglutinability between strains of these two groups. *Pf. mallei* was likewise agglutinated by both *Brucella* and *Pasteurella* sera.

Emmel and Boevers (1932) found that the sera of fowls which had been experimentally infected with *Past. aviseptica* frequently agglutinated *Br. abortus*, while the sera of fowls which had been experimentally infected with *Br. abortus* always agglutinated *Past. aviseptica*. In fact, agglutinins for *Past. aviseptica* were more frequently present in the sera of fowls that had been infected with *Brucella* than in those that had been infected with *Pasteurella* strains.

Nicolle and Comte (1910) reported the presence of agglutinins to *Br. melitensis* in the serum of forty-five out of sixty-eight patients suffering from typhus fever in Tunis. Of the reacting sera twelve agglutinated at 1/10, eighteen at 1/20–1/50, fifteen at 1/50 or over, and one at 1/100. On the basis of these results they recommended that an agglutination test with *Br. melitensis* should be carried out in the routine diagnosis of typhus fever. Numerous workers (for references see Felix (1931)) have failed to substantiate these findings, and in view of the frequency of undulant fever in Tunis and the absence of a control group of non-typhus sera, little attention can be paid to them.

In an investigation into undulant fever in Latvia, Darsin (1930) cultivated a strain of *Proteus* (not related to X 19), from an abscess in a woman's foot, which was agglutinated by human sera to the same titre as *Br. abortus*.

Jacobitz (1930) obtained a positive Weil-Felix reaction with the sera of six patients suffering from undulant fever. He states that Schnauder had observed a similar reaction in cows after abortion.

Heymann and Yang (1932), working with human, bovine and equine sera, found that sera containing agglutinins to *Br. abortus* or *Br. melitensis* frequently agglutinated *Proteus* X 19 to 1/50, 1/100, or even higher. Living suspensions, both of *Brucella* and of *Proteus*, were used for agglutination. The serum of one typhus patient that agglutinated *Proteus* X 19 to 1/200 agglutinated *Br. abortus* to 1/400.

Magliulo (1933) examined the sera of sixty patients in the early stage of tuberculosis, mostly of pulmonary type, and found that twenty-one of them agglutinated *Br. melitensis* to a titre of 1/50–1/150. Twenty of the same sera were also tested against *Br. paramelitensis*; nine of them reacted at a titre of 1/50–1/150. It was concluded that the tubercle bacillus contained a group antigen in common with *Br. melitensis* and *Br. paramelitensis*. It may be remarked that the author apparently took no steps to ascertain whether his *melitensis* strains were smooth. That he was probably dealing with non-specific agglutination is suggested by the fact that a number of sera from patients with non-tuberculous diseases, such as endocarditis, psoriasis, puerperal fever, syphilis, carcinoma, cirrhosis and lymphogranuloma, also agglutinated *melitensis* to a titre of 1/100 or 1/150.

Against these positive findings may be set the observations of workers who have failed to obtain any evidence of antigenic relationship between the various groups of organisms mentioned.

Knoth (1930) tested the sera of seven veterinarians. All the sera agglutinated *Br. abortus* to 1/75–1/100, but none of them agglutinated *Proteus* X 19.

Zeller (1931), who has had a very considerable experience of undulant fever in Germany, was unable to confirm the existence of a serological affinity between the *Brucella*, *Pasteurella* and *Pfeifferella* groups, maintained by Mallmann (1930).

Süpfle and Hofmann (1932), during an examination of 1597 human sera for antibodies to the *Brucella* group, never observed any cross-agglutination with typhoid, paratyphoid B, or dysentery strains. Nor did sera from patients infected with these three organisms agglutinate *Brucella* strains. Eleven sera containing *Brucella* agglutinins were specially tested against *Proteus* X 19 and other strains of *Proteus*, but no agglutination was observed.

Köbe (1933) examined the serum of pigs with swine fever for agglutinins to one strain of *Proteus* X 19 and two strains of *Proteus vulgaris*. Agglutinins to X 19 were not usually present, but occasionally titres of 1/10–1/100 were observed. Agglutinins to *Proteus vulgaris* were nearly always present in a titre of 1/10–1/500, most usually 1/50–1/100. Examination, however, of normal pigs, and of pigs suffering from other infections, yielded similar results. The author concluded, therefore, that the agglutinins to *Proteus* X 19 and *Proteus vulgaris* were not the result of infection with the swine-fever virus.

Priestley (1933) endeavoured to confirm the findings of Mallmann (1930) and of Emmel and Boevers (1932), but without success. Eleven sera prepared by injecting rabbits with different strains of *Pasteurella* were tested against twenty different strains of *Brucella*, in dilutions ranging from 1/2 to 1/320; no trace of agglutination was observed. Similarly three *Brucella* sera prepared in rabbits failed to agglutinate any of forty different *Pasteurella* strains. Again, no cross-agglutination was observed when two *Brucella* and two *Pasteurella* sera prepared in hens were tested against ten *Brucella* and twelve *Pasteurella* strains.

Morellini (1933) examined the sera of sixty patients suffering from pulmonary tuberculosis. The sera were put up in final dilutions of 1/100 to 1/1000 against recently isolated strains of *Br. abortus* and *Br. melitensis*, readings being made after 4–12 hours at 37° C. The results were completely negative. The author attributes the positive findings of previous workers either to the use of unsuitable strains for agglutination or to a concomitant infection with *Brucella*.

Riding (1934), working in Egypt, examined the sera of fifty typhus fever patients. All of them agglutinated *Proteus* OX 19, generally to a titre of over 1/1000, but only one agglutinated a *Brucella* suspension, the titre to *abortus* being 1/125 and to *melitensis* 1/50. Since previous work had shown that 1.7 per cent. of normal sera in Egypt agglutinate *Brucella* to 1/50 or over, this observation is without significance. No evidence was therefore obtained that patients suffering from typhus fever develop antibodies to *Brucella*.

EXPERIMENTAL WORK

Testing of horse sera for agglutinins to Br. abortus, Pf. mallei, and Proteus X19

Sixty-four horse sera from the Islington abattoir were kindly supplied by Dr F. C. Minett of the Royal Veterinary College. The exact age of the horses from which they were derived was not known, but the majority of them were probably over seven years old. Each serum was tested against a strain of *Br. abortus*, three strains of *Pf. mallei*, and one strain each of a motile (HO) and a non-motile (O) variety of *Proteus* X19. The strain of *Br. abortus* was isolated from a human case of undulant fever in 1929. It is still quite smooth, grows only in the presence of CO₂, and agrees in every particular with the bovine type. One of the strains of *mallei* (Jaques) was isolated from a human patient about eight years previously; the other two strains were isolated from horses, one in India and one in Egypt, in the course of the last year or two. Suspensions of the *abortus* and *mallei* strains were prepared by washing off 2-3 day liver agar slope cultures with 0.25 per cent. formalised saline, and heating to 60° C. for 1 hour. The *Proteus* strains, on the other hand, were used in the living state. All suspensions were standardised to an opacity corresponding to 1000 million *coli* per c.c. Agglutinations were carried out in Dreyer tubes. In fifty of the sera the final dilutions were 1/20-1/640, while in fourteen of the sera they were 1/40-1/2560. The *abortus* and *mallei* tubes were incubated in a 55° C. water bath and were read after 18 hours. The *Proteus* tubes were incubated for 2 hours in a 37° C. water bath, and were read after 16 hours at room temperature. The readings were made against a strongly illuminated dark background, using a $\times 2\frac{1}{2}$ lens, and the interpolation table worked out by Wilson and Miles (1932) was used for estimating the end-point after inversion of the tubes. The results are shown in Table I.

Table I. *Horse sera. Agglutination of Br. abortus, Pf. mallei and Proteus X strains*

	Group I 41 sera not agglutinating <i>Br. abortus</i>			Group II 23 sera agglutinating <i>Br. abortus</i>		
	Minimum titre	Maximum titre	Geometric mean titre	Minimum titre	Maximum titre	Geometric mean titre
<i>Br. abortus</i>	<20	<20	<20	20	1280	109
<i>Pf. mallei</i> *	104	832	266	160	832	315
<i>Proteus</i> HX19	<40	224	48	<40	160	36
„ OX19	<40	320	92	52	448	150

* *Pf. mallei* was tested against only 37 sera in Group I and 13 sera in Group II.

To economise space the readings with two of the *mallei* strains have been omitted, and that strain (Jaques) giving the least agglutination has been chosen. The readings with the most agglutinable strain were, on an average, rather more than twice as high as those with Jaques. The sera have been divided into two groups, Group I containing those sera which failed to agglu-

tinate *Br. abortus* at 1/20, and Group II those sera which agglutinated *Br. abortus* to 1/20 or over. Minimum and maximum titres, and the geometric mean titre, are recorded. (In working out the geometric means, sera failing to agglutinate in the lowest dilution, have been assumed to have a titre of 1/1. If all sera had been tested in an initial dilution of 1/20, the geometric mean titres, especially to the *Proteus* HX strains, would probably have been considerably higher.)

It will be noticed that every serum agglutinated *mallei*. Each of the fifty sera tested in a 1/20 dilution agglutinated *Proteus* HX19 and *Proteus* OX19, but of the seventeen sera tested in an initial 1/40 dilution, five failed to agglutinate HX19 and one OX19. The mean titres to *mallei* and to OX19 in Group II sera were rather higher than those in Group I sera. Several sera with high titres for *abortus* had quite low titres for OX19, and *vice versa*.

It may be noted that the agglutination of the *Proteus* X organisms appeared to be mainly of the somatic type. In no instance was the HO variant of X19 agglutinated to a higher titre than the O variant. The average titre to the O variant was considerably higher than that to the HO variant. This is not surprising in view of previous work which has shown that in sera from man and rabbits the ratio of the titres for the O and HO variants is about 2:1 (Felix, 1930).

*Testing of bull and cow sera for agglutinins to Br. abortus
and Proteus X strains*

Ten sera from bulls and forty-three from cows, which had been sent in to the Royal Veterinary College for examination against *Br. abortus*, were kindly supplied by Dr F. C. Minett. They were tested, in an initial dilution of 1/20 or 1/40, in the same way as the horse sera already described, but the antigens were altered. The same *abortus* strain was used; the *mallei* strains were omitted; and six *Proteus* X strains were included. The *abortus* tubes were incubated for 18 hours at 55° C., the *Proteus* X tubes for 18 hours at 37° C., both in water baths. The results are given in Table II.

Table II. *Cattle sera. Agglutination of Br. abortus and Proteus X strains*

	Group I 32 sera not agglutinating <i>Br. abortus</i>			Group II 21 sera agglutinating <i>Br. abortus</i>		
	Minimum titre	Maximum titre	Geometric mean titre	Minimum titre	Maximum titre	Geometric mean titre
<i>Br. abortus</i>	<20	<20	<20	20	53, 248	566
<i>Proteus</i> HX 2	<20	112	25	<40	208	27
„ OX 2	52	320	120	56	448	178
„ HX 19	<20	80	12	<20	160	13
„ OX 19	28	160	68	<40	208	64
„ HXK	<40	80	28	<40	208	24
„ OXK	<40	208	52	<40	208	48

Most of the *Proteus* X strains were agglutinated in a titre of at least 1/20 or 1/40. As with the horse sera, the titre to the non-motile O variants was considerably higher than to the normal motile HO forms. This suggests that

the predominant antibody in the serum of normal horses and cattle is of the O type. The results with the bull sera did not appear to differ significantly from those of the cow sera. With the exception of OX2, the average titres to *Proteus* X strains were very much the same in Group I as in Group II sera.

It is interesting to note that in horse sera the average titre to OX19 was considerably higher in those sera which agglutinated *Br. abortus* than in those which did not, while in cow sera no such difference was apparent. Furthermore, from a comparison of Tables I and II it will be seen that the average titres of Group II horse sera to *abortus* and OX19 were 1/109 and 1/150 respectively, whereas in Group II bovine sera the corresponding titres were 1/566 and 1/64. That is to say, the titre to OX19 was, on an average, higher in horse sera having a low titre to *Br. abortus* than in bovine sera having a high titre to this organism. It seemed very unlikely, therefore, that the higher average titre to *Proteus* X strains in the Group II than in the Group I horse sera could be due to the existence of a common antigenic factor between *Brucella* and *Proteus* X strains.

Many sera having high titres for *abortus* had quite low titres for *Proteus* X, while a large number of sera agglutinating *Proteus* X had no action whatever on *abortus*. Thus eight of the sixty-four horse sera agglutinated *Proteus* OX19 to a titre of 1/160–1/320, and sixteen of the fifty-three bovine sera to a titre of 1/80–1/160, without having any agglutinating action at all on *Br. abortus*.

An attempt was therefore made in Table III to obtain a more exact measure of the relation between the titres to *abortus* and OX19, and to *abortus* and *mallei*. The χ^2 values were worked out from fourfold tables, while the coefficients of correlation (r) were calculated in the usual way, using the logarithms of the titres instead of the actual figures. This measure (r) is not altogether satisfactory when applied to the present data, since the absence of observations in dilutions below 1/40 or 1/20 makes a considerable difference to the geometric mean. On the whole, however, the figures for r agree fairly well with those for χ^2 , and are sufficient for the purpose.

Table III. *Giving χ^2 and correlation coefficient (r) values.*

	Horse sera		Cow sera	
	χ^2	r	χ^2	r
<i>abortus</i> and <i>Proteus</i> OX19	7.312 $p=0.0069$	0.336 $\pm$ 0.111	1.85 $p=0.174$	-0.0107 $\pm$ 0.137
<i>abortus</i> and <i>Proteus</i> OX2	—	—	4.30 $p=0.038$	+0.4614 $\pm$ 0.108
<i>abortus</i> and <i>mallei</i>	0.008 $p=0.94$	-0.225 $\pm$ 0.134	—	—

So far as the relation between the titres of horse sera to *abortus* and *mallei* is concerned the χ^2 value is negligible, and the value of r is not only low but is negative. With *abortus* and OX19, however, the figures are more puzzling. The value of χ^2 for horse sera is fairly high, indicating that such a distribution of titres to the two organisms, provided that these were antigenically independent, would not be likely to occur by chance alone more often than once

in 145 times. This is, moreover, borne out by the figure of $+0.336$ for r , which suggests that there is a definite correlation between the antibodies for the two organisms. On the other hand, in cow sera, χ^2 is very low, and r is not only small but is negative. Since both the horse and the cow appear to respond readily to *abortus* infection, it is difficult to understand why, if there is a real antigenic relationship between *abortus* and OX19, the correlation between the titres to the two organisms should be so high in horse sera and so low in cow sera.

Unfortunately the horse sera were not tested against OX2, but the cow sera exhibited much the same peculiarity towards this organism as the horse sera did towards OX19; that is, the average titre was higher to OX2 in those sera which agglutinated *abortus* than in those which did not.

No simple explanation for these phenomena suggests itself. It may be that the animals from which Group II sera were derived were rather older than those of Group I, and, as Lovell (1932) and Jordan (1933), and others have shown, normal agglutinins are much more frequent in older than in younger animals. But this does not explain why in the horse sera the titre to HX19 was lower, and the titre to OX19 higher, in Group II than in Group I sera. Nor does it explain why OX2 was the only one of the six *Proteus* variants tested that showed a higher titre in Group II cow sera; nor again why the titre to OX19 was higher in Group II than in Group I horse sera, while in cow sera the titres in the two groups were almost identical. The only conclusion that can safely be drawn is that, in view of these various discrepancies, any attempt to deduce antigenic relationships from the co-existence in the same serum of antibodies to two different organisms must be regarded with misgiving.

*Cross-agglutination and cross-absorption experiments with
Brucella and Proteus X19*

In order to gain more direct evidence on the possible antigenic relationship of *Brucella* and *Proteus* X strains, cross-agglutination and cross-absorption experiments were carried out with a number of different sera. Reproduction of the protocols of these experiments would take up an unjustifiable amount of space, and since the results were perfectly clear-cut, they may be summarised as follows:

(1) Antisera prepared in the rabbit against *Proteus* HX2, HX19, HXK, OX19 (two sera), and OXK, with O titres to their homologous strains varying from 1/6400 to 1/32,000, were tested in final dilutions of 1/20–1/640 or 1/40–1/1280 against one bovine *abortus*, one porcine *abortus*, and two *melitensis* strains. No agglutination whatever was observed.

(2) The same six antisera were absorbed in dilutions of 1/50–1/200, depending on the titre, with enormous doses—150,000 million organisms per c.c.—of one strain of bovine *abortus* and one of *melitensis*. No significant lowering of the titre was observed in any instance. The only alteration that

was noticeable was a diminution, not in the titre, but in the amount of agglutination, when certain OX sera were absorbed with a *melitensis* suspension, and tested against an HX strain; the titre to the homologous OX strain remained unchanged. The reason for this interference with agglutination of the somatic antigen in flagellated organisms is not clear. It was thought that it might be an instance of that type of non-specific absorption by unwashed bacterial bodies described by Weil (1911), but absorption experiments carried out with washed and unwashed *Brucella* organisms failed to support this supposition. More probably it was connected with the formol used for preserving the *Brucella* suspensions. Felix and Olitzki (1928), it will be remembered, drew attention to the inhibitory effect of formol on the reaction between O antibody and O antigen that was observed when flagellated bacteria were employed as the agglutinating suspension. More recently Craigie (1931), who has studied the phenomenon, has brought strong evidence to suggest that it depends on the fixation by the formol of the bacterial flagella, the distribution of which around the organisms prevents the latter from coming into close contact with each other. In the present work direct experiment showed that the inhibition of somatic agglutination in flagellated *Proteus* X strains occurred only when the absorbing *Brucella* suspensions contained formol (0.25 per cent.).

(3) Seven bovine *abortus*, five porcine *abortus*, and nine *melitensis* antisera prepared in the rabbit, and one serum from a horse naturally infected with *Br. abortus*, were absorbed in a dilution corresponding to 1/32nd of the titre with 10,000 million per c.c. *Proteus* HX 19 and OX 19. No lowering of the titre for the homologous strain was ever observed.

These experiments afford no evidence that there is any antigenic relationship between *Brucella* and *Proteus* X strains.

Absorption of Brucella sera by Pfeifferella strains

One bovine *abortus* and one *melitensis* antiserum prepared in the rabbit were absorbed at 1/32nd of the titre with 10,000 million *Pfeifferella* organisms—a dose three times that necessary to remove the agglutinins when the homologous strain was used for absorption. One porcine *abortus* antiserum was absorbed with 3000 million *Pfeifferella* organisms. Four strains of *Pf. mallei* and one strain of *Pf. whitmori* were used separately for absorbing each serum. No reduction in titre for the homologous organism was ever observed.

Absorption of Pfeifferella sera by Brucella strains

Three horse sera agglutinating *mallei* to between 1/100 and 1/450 were absorbed in a 1/5 dilution with 10,000 million *abortus* and *melitensis* organisms. In this dose *mallei* removed all the agglutinins from the sera, while neither *abortus* nor *melitensis* had any effect on the titre to *mallei*.

Miscellaneous cross-agglutination tests

Antisera prepared in the rabbit against strains of porcine *abortus*, *bronchiseptica*, *Bact. faecalis alkaligenes*, *Bact. typhosum*, *Bact. enteritidis* O (Gärtner),

Bact. flexneri, V, W, X and Z, *Bact. shigae*, *Pf. mallei*, and *Pf. whitmori* were tested each in dilutions of 1/20–1/640 against some or all of four *Brucella*, two *bronchiseptica*, one *faecalis alkaligenes*, one *typhosum*, one *enteritidis* O, four *flexneri*, one *shigae*, two *morgani*, one *Proteus* X19, one *mallei*, and one *whitmori* strain. With the exception that the porcine *abortus* serum, whose titre was 1/1280, agglutinated *flexneri* X and *whitmori* in a 1/20, *flexneri* Y in a 1/40, and one strain, but not another, of *mallei* in a 1/80 dilution, no cross-agglutination was observed. Since agglutinins to these organisms are normally present in many sera, little attention need be paid to the slight degree of agglutination that occurred. It has moreover been shown that *Pfeifferella* strains are unable to absorb the agglutinins from *Brucella* sera.

DISCUSSION

The experiments recorded in this paper, taken in conjunction with those of Knoth (1930), Zeller (1931), Süpfle and Hofmann (1932), Köbe (1933), Priestley (1933), Morellini (1933), and Riding (1934), lend no support to the suggestion put forward by various workers of the existence of an antigenic relationship between *Brucella* strains on the one hand, and strains of *Pfeifferella*, *Pasteurella*, and *Proteus* X on the other. The evidence on which such an assumed relationship is based is wholly insufficient to bear the weight of the conclusions drawn from it. Most of the observations that have been made on the coagglutination by a given serum of two or more groups of bacteria can be explained as a result of the presence in the serum of natural agglutinins to one organism together with acquired agglutinins to another organism, or as the result of the presence of acquired agglutinins to both organisms, or occasionally as a result of the use for test purposes of a strain that is readily agglutinated by non-specific agencies. The co-existence of different agglutinins is by no means uncommon, and it cannot be held to indicate any antigenic relationship between the organisms unless supported by careful cross-absorption experiments.

The evidence accumulated in the past few years points very strongly in favour of the existence of a high degree of antigenic specificity in most groups of organisms examined. In the interpretation, however, of routine agglutination tests made on human or animal sera, it is essential to know the normal level and variation of agglutinins, either naturally present or acquired through latent or overt infection, to the particular organism under consideration. It is neither generally nor sufficiently recognised that the level of "normal" agglutination for a given species depends directly on the sensitiveness of the strain used in estimating it. In somatic "O" agglutination, with which the present paper is mainly concerned, the difference in sensitiveness of different strains of the same species is very considerable. One of the best-known examples is that of strain 901 of *Bact. typhosum*, whose sensitivity to O agglutinins is five to ten times that of the common strains of this organism (Felix, 1929).

A further point,* which cannot be too strongly emphasised, is the necessity

in routine work for selecting antigenically smooth strains for the preparation of agglutinating suspensions. Strains that are in process of becoming antigenically rough frequently display an anomalous behaviour which is apt to be very confusing. Thus they may agglutinate to only a fraction of the titre recorded with a smooth strain, or, on the other hand, they may be so hyper-sensitive as to react to an unduly high titre with a smooth antiserum, or, what is even more misleading, they may show frank non-specific agglutination.

SUMMARY AND CONCLUSIONS

1. Provided that due precautions are taken to ascertain the normal level of agglutinins in a given host, and that antigenically smooth strains are used, there is every reason to believe that the occurrence of agglutinins in a titre above the normal range of variation is due to infection—latent, active or past—with the specific organism in question, or in a few instances with an organism, usually of the same genus, sharing a similar antigen.

2. This conclusion may have to be modified for the occurrence of agglutinins to *Proteus* OX19 in human typhus sera, since the exact relationship of this organism to *Rickettsia prowazeki* is still doubtful. If, however, future work shows the truth of Felix's (1933) contention that serological types of *Proteus* X correspond to serological types of typhus virus (*Rickettsia*), even this apparent exception will fall within the general rule.

3. The examination of sixty-four horse and fifty-three cattle sera, and the performance of numerous cross-agglutination and cross-absorption experiments with rabbit and horse antisera, lend no support to the suggestion made by certain workers of the existence of an antigenic relationship between *Brucella* strains on the one hand, and strains of *Pfeifferella*, *Pasteurella* and *Proteus* X on the other.

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