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The antigens of *Trichomonas foetus* isolated from cows and pigs

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INTRODUCTION

The strains of *Trichomonas* studied were *T. foetus* strain *Belfast* (*B*) isolated by Dr Kerr in Belfast in 1938 and strain *Manley* (*M*) isolated by Mahmoud (1944) in Liverpool, both from the genital tract of infected cows. Two strains (S 2 and *414*) isolated from the caecum of pigs in Munich were kindly placed at my disposal by Drs Hammond and Leidl in 1957*; they have a morphology similar to that of strains *B* and *M* but are a little more difficult to maintain in culture.

The investigation was primarily concerned with the antigenic structure of the cow strains *B* and *M* and secondly with their relation to the two pig strains.

MATERIAL AND METHODS

Maintenance of strains

The strains were maintained on the 'diphasic' medium of Kerr & Robertson (1953), modified by substitution of peptone Lemco glucose broth for the tryptic digest glucose broth. For bulk growth the medium was enriched with 7 % horse serum and sterilized by filtration through a Seitz filter.

Preparation of antisera

Antisera were made in rabbits by the intravenous injection of living washed organisms suspended in 0·85 % saline. Protozoa are not very good antigens; dried antigens produced very poor results and were not used. Five bi-weekly injections, starting with doses of 100–200 $\times 10^6$ organisms, rising to 1000–1200 $\times 10^6$ at the finish, produced sera good for agglutination tests but inadequate for gel-diffusion tests. Good precipitating antisera were produced by a second course, starting 4–8 weeks later, of four or five injections, beginning with doses of 50–100 $\times 10^6$ organisms and finishing with 1000 $\times 10^6$; on occasions a short third course was given. Good sera were also produced by a single course of ten bi-weekly injections reaching 1200 $\times 10^6$ by the fifth dose and remaining at this level for the remaining five injections. This method, while it saved time, was not as a rule so well tolerated by the rabbit.

Agglutinable suspensions

Agglutination tests were made with live organisms (Robertson, 1941; Pierce, 1947). Strains of *Trichomonas* are not all equally agglutinable and this is particularly true of recently isolated ones. The pig strains were a little irregular in this

* Dr Hammond has since informed me that strain *414* was isolated from the caecum of a pig in Utah, U.S.A.

respect when first grown in the medium used; their rate of reproduction and their motility in the test suspensions was variable. After some months subculture they were quite serviceable in agglutination tests. Important points in carrying out the tests are: the suspensions should be made in broth saline at pH 7·0 to 7·4 and for optimum results the number of organisms should be 100,000/ml. in the suspension to be added to the antiserum. All antisera, even those which have been stored at −10° C. for considerable periods, should be heated once to 56° C. for 20 min. before use. Agglutination results are expressed as the reciprocal of the titre at which '+' agglutination (Pierce, 1947) occurs.

Antigen preparations for precipitin and gel-diffusion tests

(*a*) '*Total antigen*'. The trichomonads obtained by spinning down fresh 48 hr. cultures were disintegrated in a Townson & Mercer 'Macerator', and the resulting material freeze-dried after dialysis. For use, the dry mass was extracted overnight with 0·85% saline at 2° C. and insoluble matter removed by centrifugation until the supernatant fluid was quite clear. For gel-diffusion tests, however, it was not necessary to have a completely clear solution.

(*b*) '*Protein*' *fractions*. Live organisms concentrated from culture by centrifugation were alternately frozen in 0·85% saline at −80° C. and thawed. The cellular debris was removed by centrifugation, and the fluid dialysed against distilled water for 48 hr. at 2° C. Four fractions were precipitated from the dialysate at −6° C. by ethanol: TF 1 precipitated by 20% (v/v) ethanol; TF 2 by 40%; TF 3 by 60% and TF 4 by 80%.

(*c*) '*Polysaccharide*' *fraction*. The extraction of *T. foetus* strain *M* with anhydrous diethylene glycol yields a serologically active substance composed of a polysaccharide and an amino-acid moiety (Feinberg & Morgan, 1953) which is not immunogenic but precipitates with homologous *M* antisera, and which (Kerr, McGirr & Robertson, 1949; Kerr & Robertson, 1952, 1953) elicits skin reactions in cattle infected with *T. foetus*. Diethylene glycol extracts of this kind were made of the *M* and the *B* strains.

Precipitin tests

Constant-antibody titrations were carried out with antisera diluted either 1/1 or 1/2, and 'total antigen' preparations in doubling dilutions up to 1/32. Readings were made after 48–72 hr. at 10° C. The precipitate in each tube was assessed numerically, with a maximum score (++++) = 4 and the degree of precipitation in the series expressed as the total of all the readings (see Table 2). This value is a truer measure of the precipitability of the antigen than end-point readings, which in any event are difficult to make precisely in a twofold dilution series of the antigens.

Gel diffusion

High-titre antisera were tested in Ouchterlony plates (1948, 1953) against all three kinds of antigen preparations.

RESULTS

Agglutination and precipitation

The results of agglutination tests with all four strains are summarized in Table 1. The serological distinction between the strains *B* and *M*, first shown with antisera from infected cattle (Kerr & Robertson, 1945) was confirmed by Pierce (1949) in a field survey of the strains causing outbreaks of the disease in England. The distinction was also very clear with rabbit antisera, which agglutinate the homologous and heterologous suspensions to substantially different titres. Thus (Table 1) antisera with titres of 3000–6000 against the homologous *B* strain agglutinated strain *M* only to 48–192; and conversely with *M* antisera.

B antisera cross-reacted strongly with the pig strains *S2D* and *414* to titres of 768–1536. *M* antisera reacted somewhat less well with the pig strains, the titres with *S2D* being about 384 and 768 with strain *414*.

Antisera to the pig strain *S2* agglutinated both the *M* and the *B* cow strains—a result that might occur if *S2* were a mixture of two strains, one with *M* and the other with *B* specificity. Three single-cell culture (clones) of *S2* kindly isolated by Dr Pierce were investigated to test this possibility. All three reacted like the parent strain with *S2* antiserum, and antisera to the clone strains reacted like the original *S2* antiserum. *S2* was used (Kerr, 1958) to infect a heifer at Stormont. The passage strain from this animal was agglutinated by *S2* serum exactly as the original strain and as the three clones. The passage strain was not maintained.

S2 antisera agglutinated the homologous strain to titres of 3000–6000, the heterologous strain *414* to titres of 1500–3000, and the *B* and *M* strains to about 768–

Table 1. *End-titres of cross-agglutination reactions of* Trichomonas *antisera and suspensions of live trichomonads*

	Antigen			
Antiserum	*B*	*M*	*S2**	*414*
B	3000–6000	48–96	768–1536	768–1536
M	48–192	3000–6000	384	768
*S2**	768–1530	768–1530	3000–6000	1500–3000
414†	768	24–48	1536	3000

* Characteristic of the agglutinability of the original strain, three clones derived from it and the strain after passage through an infected heifer.

† Antiserum induced by a short course of immunization.

Table 2. *Degree of cross precipitability of 'total' antigen preparation of trichomonads with* Trichomonas *antisera*

	Antigen			
Antiserum	*B*	*M*	*S2D*	*414*
B	**13·5**	5	7·5	9
M	4	**14·5**	3	3
S2	10	6·5	**14**	10·5
414	11	5	11	**14·5**

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1536. A *414* antiserum induced by only five injections of antigen distinguished the four strains very clearly; the homologous titres were 3000, 1536 for *S2*, 768 for the *B* strain and only 24–48 for the *M* strain (Table 1).

The results of the precipitin tests in Table 2 indicate the relations between the four strains and are consistent with the agglutination results. The cow strains *B* and *M* are readily distinguishable; and the two pig strains are very closely related. The *B* antiserum reacts better than *M* antiserum with the pig strains.

Gel diffusion

Reactions of cow-strain antigens

Gel diffusion plates revealed a complex antigenic make up of the trichomonads, indicated by at least five lines. No attempt was made to identify all the individual lines. With antisera to living organisms and 'total antigen', the lines produced by homologous strains *B* and *M* could be arranged in groups. The five lines were numbered from the antiserum towards the antigen cup. Lines 1–3, distinct in certain plates, were very often fused in others. Line 4 was very distinct with certain sera and was often double. Nearest the antigen cup came line 5, a complex which was certainly double and may have represented several lines.

The polysaccharides of strains *M* and *B*, as extracted by diethylene glycol, were serologically specific for the strains from which they were obtained. Their lines were not shared in the diffusion plates, but lines other than those of the polysaccharide complex were shared by both strains. With a *B* antiserum, total antigen from *M* produced only non-polysaccharide lines, whereas *B* polysaccharide produced only the polysaccharide lines. The polysaccharide lines of *M* and *B* corresponded to line 5 produced respectively by total *M* and *B* antigens (Fig. 1).

It was considered probable that the lines which lay between the serum cup and the polysaccharide lines in a system with the total antigen and which were shared by both the strains represented protein reactions. Supporting evidence was obtained by a different approach. The fraction TF 4 from strain *B* appeared to be mainly protein. In a diffusion plate with *B*-serum fraction TF 4 produced no polysaccharide lines, but clear lines continuous with non-polysaccharide lines of the total antigen from *B*. A neutral protein fraction (NP) was isolated from the total *B* antigen by electrophoresis on the starch column, which did not migrate at pH 7·0. Against *B* antiserum it reacted like TF 4, without any polysaccharide lines. The TF 4 lines corresponded to the 1–3-line complex and usually also to line 4. The same sets of protein lines were produced with antisera to the *M* and the two pig strains.

Precipitation of enzyme preparations from T. foetus *strain B*

It was of some interest to determine the antigenic properties of two well-defined substances from *T. foetus*.

Two enzyme preparations which destroyed the serological activity of blood group B and H substances, respectively, were isolated by Dr Watkins (1959) from *T. foetus* strain *Belfast* by zone electrophoresis on a starch column. With antisera to strain *Belfast* the B-hydrolysing enzyme gave an extremely feeble polysaccharide

line but no protein lines. The H-hydrolysing enzyme preparation in contrast gave both a polysaccharide complex and the 1–3 protein lines corresponding with those of the total antigen. One H-enzyme preparation had no line 4 and so could be used to identify line 4 in other antigens by its abrupt termination at the enzyme cup. A second H-enzyme preparation had line 4, owing probably to the slightly different conditions under which the electrophoresis was carried out. The B- and H-enzyme preparations were equally potent in their action on the appropriate blood group substrates. It was concluded that the lines in the diffusion plates produced by enzyme preparations were not due to the enzymes themselves, but to other antigens separating in the same electrophoretic zone.

The independence of enzymes and the antigens responsible for the gel-diffusion lines is confirmed by the reactions of the 'protein' fractions. Of the T series, TF 3 had the greatest enzyme activity against blood-group substances, and gave a full complement of lines with a *B* antiserum.

Reactions of pig-strain antigens

The *414* and *S2* antigens, tested in parallel with those of *B* and *M*, gave strong cross-reactions corresponding to those revealed by agglutination and tube precipitation. The general disposition of the lines corresponded with the 1–3, 4 and 5 complexes already found with the cow strains. The protein lines of all the four strains corresponded, and were continuous with each other in reactions of the antisera to all of the four varieties of trichomonads. However, the polysaccharide lines were only partially shared, often having a free 'spur' in addition to the continuous lines, suggesting an element in the polysaccharide complex which was distinct (Ouchterlony, 1953).

The TF 4 fraction of the *B* antigen reacted with *S2* and *414* antisera as it did with the *M* and the homologous *B* antisera. Its protein lines appeared with all the sera and there were no polysaccharide elements (Figs. 2, 3).

DISCUSSION

All the four strains are closely similar as regards their protein antigens. In the two cow strains *B* and *M*, there is a clear distinction between their polysaccharide antigens, although with high-titre antisera produced by long immunization courses there was a varying amount of cross-reaction. The two pig strains are very closely related to each other, but the degree of correspondence of the agglutination and the precipitation results does not amount to serological identity. The *B* strain reacts to a greater degree than the *M* strain with antisera made from the pig strains.

Hammond & Leidl (1957) and Fitzgerald, Johnson, Thorne & Hammond (1958) demonstrated that the *Trichomonas* from the intestine of the pig can infect the genital tract of the bull, which in turn can transmit the infection to the cow at coitus. Kerr (1958) also produced infections with one of Leidl and Hammond's strains (*S2*) and with a strain (*Stormont*) that he isolated from pig faeces in Belfast. In an agglutination test with the vaginal mucin from a heifer infected with the

Stormont strain he found that the *B* cow strain and the *Stormont* pig strain were agglutinated to a titre of 160, whereas the *M* strain was agglutinated only to 10. The agglutination titre of the vaginal mucin of an infected cow is always low, but it is the best index of the presence of infection for use in the field.

There seems to be no valid ground for distinguishing as separate species the strains isolated from the caecum of the pig by Leidl and Hammond and the strains found in the uterine infection of the cow.

SUMMARY

The serological properties of the two strains of *Trichomonas foetus* (strain *B* and strain *M*) isolated from uterine infections in cows in Belfast and in northern England, respectively, and two strains isolated from the caecum of pigs were investigated with rabbit antisera to each of the four strains by direct agglutination and by precipitation of a saline extract of the dried organisms in tubes and in gel-diffusion plates.

There was considerable cross-reaction between the four strains. By the use of predominantly polysaccharide fractions from strains *B* and *M* and the predominantly protein fraction TF 4 from strain *B*, the polysaccharide complex of lines could be distinguished from the protein lines.

The major protein antigens were common to all four strains, but the major polysaccharide antigens were only partially shared.

The cow strains *B* and *M* were readily distinguished from each other but had a variable though slight serological relationship. The two pig strains were very closely related to each other but were not identical. Cross-reaction revealed a relation between the cow and the pig strains. The cow strain *B* was more closely related than the *M* strain to the pig strains.

The serological distinctions do not justify the separation of the pig and the cow strains of *T. foetus* into two species.

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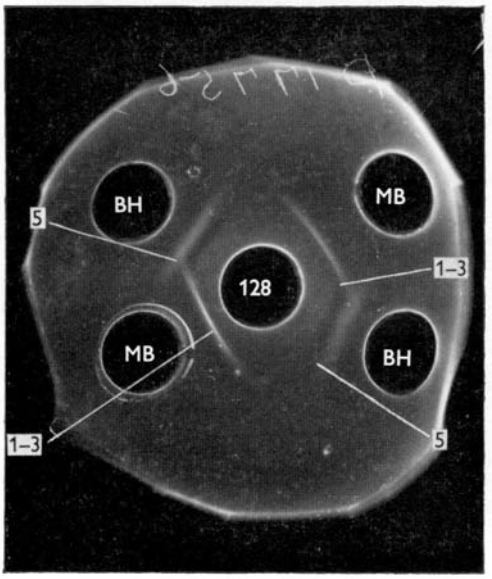

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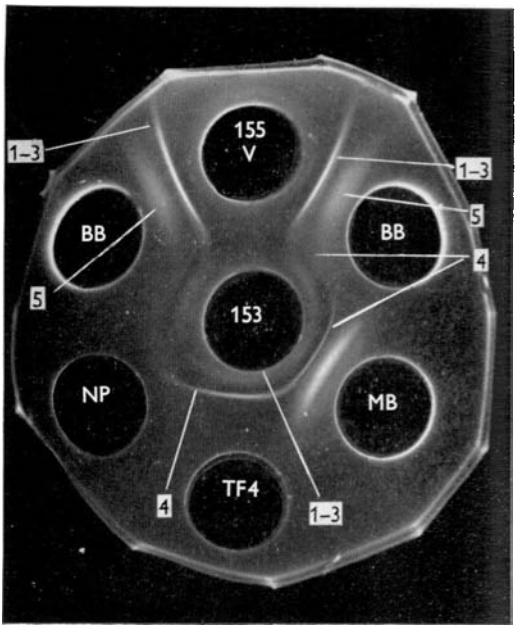

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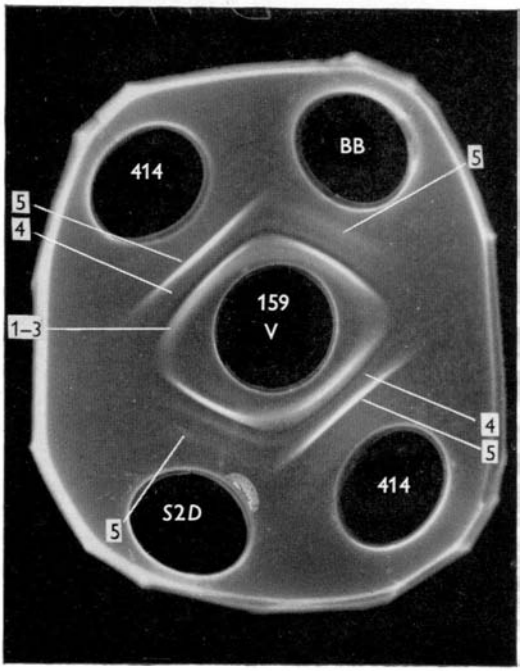

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EXPLANATION OF PLATE

Fig. 1. Ouchterlony precipitation with *B* antiserum 128 (centre cup). With BH, the polysaccharide fraction of strain *B*, only lines of complex 5 develop. With MB, the total antigen of strain *M*, only the protein complexes 1–3 develop.

Fig. 2. Ouchterlony precipitation with *B* antiserum 155 (top cup) and *M* antiserum (centre cup). BB, the total antigen of strain *B* with homologous antiserum *B* gives a strong protein complex 1–3, a faint line 4 on the right, and a diffuse band for complex 5. MB, the total antigen of strain *M*, gives an analogous set of lines (line 4 is clear) with homologous antiserum *M*. The 1–3 and 4 complexes are with varying intensities continuous round the two serum reservoirs, being shared also by TF 4 and NP, the 'protein' fraction and the 'neutral protein' fraction from strain *B*. The M and B polysaccharide lines are not shared.

Fig. 3. Ouchterlony precipitation with *414* antiserum (centre cup). The 1–3 and the 4 complexes are continuous for total antigens of strain *414*, strain *B* (BB) and strain *S2D*. The 5 complex of antigen *414* is partly shared by *B* and *S2D*, but spurs at the junction of the 5 complexes between *414* and *B*, and *414* and *S2D* indicate a substantial serological difference between the antigen 5 of the three strains.