SEROLOGICAL ASPECTS OF *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis has now been accepted as a cause of infections in the lower genital tracts of both males and females. However, it is often found in considerable numbers in the absence of any clinical symptoms or signs; it then appears to be a harmless saprophyte or commensal. This difference in behaviour has not been satisfactorily explained, although many suggestions have been put forward. Mascall (1957) regarded the difference as being due to a variable body reaction to the same organism; others have suggested that only in symbiosis with certain and as yet unidentified bacteria does *Trichomonas vaginalis* become pathogenic. A third possibility which invites exploration is that of variations, morphological or antigenic, in the organism itself. The morphology of *T. vaginalis* has, however, been widely studied and, although Feinberg (1954) described minor and possibly unimportant flagellar abnormalities, no other significant variation has been noted. Serological investigations have been less extensive, and the existence of distinct strains of the organism does not appear to have been seriously considered. If such strains existed and could be identified, they might well be of clinical interest and have an important bearing on both diagnosis and treatment. The serological studies here described were undertaken with this in mind. It must, however, be recognized that certain difficulties present themselves in this field; unlike bacteria, protozoa are poor antigens and the presence of normal agglutinins in the sera of both man and most domestic animals can be misleading.

The first recorded serological observations on *T. vaginalis* appear to have been made by Riedmüller (1932), who noted the formation of complement-fixing antibodies in guinea-pigs after intraperitoneal injections of vaginal discharge containing

the organism. Later, Tokura (1935) reported the finding of agglutinating antibodies in rabbits after repeated intravenous injections of a killed culture of the organisms. However, the bulk of serological investigations on trichomonads has been carried out by veterinary workers in their study of *T. foetus*. This work is far too extensive to be mentioned here, but of special interest was the finding of the Manley and Belfast strains of *T. foetus* in 1945. Reference must also be made to the description of serum agglutinin formation in bovine trichomoniasis (Witte, 1934), and to the development of a micro-agglutination technique using sera from artificially immunized rabbits (Robertson, 1941). Trussell (1946), employing this same technique with *T. vaginalis*, obtained satisfactory agglutination reactions but there is no record of further investigations.

The report which follows describes attempts to investigate the possibility of strain variations in *T. vaginalis* in Liverpool during the last 2 years. It was originally intended to test a number of specimens of *T. vaginalis* against an immune serum. Those which were not agglutinated by the serum were to be used for the preparation of further immune sera for cross-agglutination testing. It was hoped that, after examining a series of locally obtained specimens, others would be acquired from different parts of the United Kingdom using a transport medium. This plan, however, was not closely followed as it became apparent during the course of the investigation that additional measures were necessary.

Materials and Methods

Specimens for culture were taken from unselected male and female patients attending the Special Department of the Liverpool Royal Infirmary. The main culture medium was that of Feinberg (1954) plus 1,000 units per ml. streptomycin. The

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routine inclusion of penicillin was found unnecessary and, indeed, was thought on occasions to have an inhibitory effect on new culture growth. To combat surviving bacterial contaminants, the appropriate antibiotic was used as determined by sensitivity tests, and aerosporin (500 units per ml.) was most commonly employed. Monilial infections responded satisfactorily to the addition of methyl violet in strengths of 1—20,000 to 40,000, but this likewise appeared to have an adverse effect on the organism. For rabbit inoculations, vaccines containing approximately 20 million organisms per ml. were made. To obtain this concentration ten tubes containing 10 ml. medium were inoculated; after 30 hrs growth the tubes were kept at -2°C . for 24 hrs; the residues were then pooled, washed in saline, centrifuged, counted, and diluted accordingly.

Specimens for testing against immune sera were cultured until a population of approximately $\frac{1}{2}$ million organisms per ml. was obtained. Centrifuging of cultures to obtain this concentration proved unsatisfactory as it led to equivocal results in subsequent agglutination reactions. Because of the possibly selective nature of Feinberg's medium, an attempt was also made to isolate new specimens on a simple medium without antibiotics. For this purpose a two-phase medium consisting of Loeffler's slope, and Ringer's solution containing 1 in 8 inactivated horse serum, was used. It was intended that the growth once established should then be transferred to Feinberg's medium. Immune rabbit serum was prepared as follows:

Six intravenous injections of the suspension containing 20 million trichomonads per ml. were given in the course of 3 weeks. The doses were graduated and given twice-weekly; $\frac{1}{2}$ ml. the first week, and 1 and 1.5 ml. in subsequent weeks. In addition to these, however, it was found necessary to give a "booster" dose one week later of 2 ml. in order to produce adequate agglutinin formation.

The rabbit was bled 3 days after the last injection. Control samples of serum were taken from the rabbit before immunization for use in relevant agglutination tests. All sera were inactivated in a water bath at 56°C . for 30 min.

Technique of Agglutination Test

Serial dilutions of immune serum from 1 in 5 to 1 in 320 were set up in approximately 0.05 ml. volumes in 3 by $\frac{1}{2}$ -inch tubes. Equal amounts of the medium (containing $\frac{1}{2}$ million trichomonads per ml.) were added to each tube. Control tubes were similarly prepared. After shaking, the racks of tubes were incubated for 1 hr at 37°C . The contents were then poured gently on to a microscope slide and examined microscopically under low power. The degrees of agglutination were signified by + + + + for maximum to — for complete absence.

Course of Investigation

Efforts to isolate specimens of *T. vaginalis* on the two-phase medium were successful in nine out of ten attempts. The transfer of the organism to Feinberg's medium inevitably introduced an overgrowth of mixed bacteria which, on every occasion, proved most difficult to eradicate, and for this reason this experiment was discontinued.

Using primary inoculations of Feinberg's medium, no difficulty was met in obtaining positive cultures from microscopically positive specimens. To maintain these cultures until the organisms were numerically sufficient for testing against immune sera was more difficult. Some specimens grew extremely well from the start, and others required weeks of subculturing. For example, one specimen was cultured to the desired count in one week while another required as long as 3 months. Frequently, quite healthy cultures would fail for no apparent reason, but in general it was the specimens with poor initial growth response which proved most troublesome. The failure rate amongst these was considerable and was responsible for only 22 of 68 specimens being successfully cultured to the desired level. These cultural differences are perhaps significant but no clinical correlation was obvious.

The first Immune Serum (I.S.1) to be prepared, when tested against its antigen (A.1), showed adequate agglutinating properties (Table I) even in the highest dilutions.

TABLE I
AGGLUTINATION RESULTS OF ANTIGEN 1 AGAINST IMMUNE RABBIT SERUM 1

Rabbit Serum						Dilutions							
						1/5	1/10	1/20	1/40	1/80	1/160	1/320	Control
Immune	..	..	..	..	..	+	++	++	+++	++++	++	+	—
Control		..	..	..	..	—	+	+	—	—	—	—	—

+ + + + = maximum agglutination: — = no agglutination

The agglutination picture was not uniform throughout. In the lower dilutions the organisms were less motile and many appeared dead. No other physical changes were obvious but more prolonged observations might perhaps have been informative. The absence of effective agglutination at these dilutions possibly resulted from factors associated with this loss of motility. Conversely, it may have illustrated a prozonal effect. The most marked agglutination occurred in the middle dilutions when rosette and "bee-hive" clumps were seen. Non-agglutinated organisms and those at the periphery of the clumps appeared healthy and very active. In the highest dilutions the clumps were small and the organisms showed no loss of motility.

It is interesting to compare Table I with Table II which illustrates the amount of agglutination obtained by Immune Serum 1 against Antigen 1 before the 2 ml. "booster" dose was given.

It was appreciated that the presence of normal agglutinins in rabbit sera could falsify results. As a general guide to later investigations a group of five normal rabbit sera was tested for agglutinating powers using A.1 as antigen. The results are set out in Table III.

These results indicated that normal rabbit agglutinins might be effective in dilutions up to 1:40 and that agglutinations beyond this titre might be considered specific.

A series of twenty different specimens of *T. vaginalis* which had been cultured to the required counts was then tested against I.S.1. Eighteen of these (Table IV) were agglutinated by the immune serum at dilutions similar to the homologous strain, but two specimens remained unaffected. These negative results were obtained on repetition on three different occasions.

TABLE IV

AGGLUTINATION RESULTS OF TWENTY DIFFERENT SPECIMENS OF *T. VAGINALIS* AGAINST IMMUNE SERUM 1

Number of Specimens Tested	Agglutination	
	Present	Absent
20	18	2

These results suggested that the two non-agglutinated specimens were perhaps serologically different from Antigen A.1.

It was then decided to prepare immune sera from these and to carry out cross-testing, but both these cultures became heavily contaminated and died before this could be done. A further possibility suggested by these results was that I.S.1 contained multiple agglutinins which accounted for the high number of apparently similar serological strains. Three more immune sera, I.S.2, I.S.3, I.S.4, were therefore prepared from specimens of *T. vaginalis* (T.2, T.3, T.4) all of which were obtained from

TABLE II

AGGLUTINATION RESULTS OF ANTIGEN 1 AGAINST IMMUNE RABBIT SERUM 1 BEFORE ADDITIONAL "BOOSTER" DOSE

Rabbit Serum	Dilutions							
	1/5	1/10	1/20	1/40	1/80	1/160	1/320	Control
Immune before "Booster"	+	++	+	++	+	±	—	—
Control	—	+	+	—	—	—	—	—

++++ = maximum agglutination: — = no agglutination

TABLE III

AGGLUTINATION RESULTS OF ANTIGEN 1 AGAINST FIVE NORMAL RABBIT SERA

Normal Rabbit Serum	Dilutions							
	1/5	1/10	1/20	1/40	1/80	1/160	1/320	Control
1	—	+	+	—	—	—	—	
2	—	++	+	—	—	—	—	
3	—	++	+++	—	—	—	—	
4	—	+	+	++	—	—	—	
5	—	+	++	++	—	—	—	

++++ = maximum agglutination: — = no agglutination

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TABLE V
AGGLUTINATION RESULTS OF ANTIGENS 1, 2, 3, AND 4 AGAINST IMMUNE SERA 1, 2, 3, AND 4

Immune Serum	Antigen	Dilutions							Control
		1/5	1/10	1/20	1/40	1/80	1/160	1/320	
1	T1	+	++	++	+++	+++	++	+	—
	T2	—	—	+++	+++	—	—	—	—
	T3	++	++	++	+++	+++	+++	++	—
	T4	++	+++	+++	+++	+++	+++	++	—
2	T1	++	++	+	+++	—	—	—	—
	T2	++	+++	+++	++	+++	++	++	—
	T3	+++	+++	+++	+++	+++	+++	+++	—
	T4	+	+++	++++	+++	—	—	—	—
3	T1	+++	++	—	—	—	—	—	—
	T2	++	++	+++	++	+	++	++	—
	T3	++	++	+++	+++	+++	++	++	—
	T4	++	+++	++	—	—	—	—	—
4	T1	+++	+++	+++	++	++	++	+	—
	T2	++	+	+	—	—	—	—	—
	T3	+	++	+	±	—	—	—	—
	T4	+	+++	+++	+++	++	++	+	—

++++ = maximum agglutination: — = no agglutination

different and unselected patients. Each antigen was tested against all the immune sera and control samples of normal serum. The results are shown in Table V.

It will be seen that Immune Serum 1 contained agglutinins against Antigen T.1, 3, and 4, I.S.2 against Antigen T.2 and 3, I.S.3 against Antigen T.2 and 3, and I.S.4 against Antigen T.1 and 4. These results are summarized in Table VI.

TABLE VI
SUMMARY OF AGGLUTINATION RESULTS OF ANTIGENS T.1, 2, 3, AND 4 AGAINST IMMUNE SERA 1, 2, 3, AND 4

Antigen	Immune Serum			
	1	2	3	4
T1	+	—	—	+
T2	—	+	+	—
T3	+	+	+	—
T4	+	—	—	+

+ = agglutination: — = absence of agglutination.

These readings seemed to indicate a certain pattern of agglutinins. In this small series, three distinct groupings of four agglutinins appeared to be present. I.S.1, which had previously agglutinated eighteen out of twenty specimens of *T. vaginalis*,

apparently contained three agglutinins; I.S.2 and 3 apparently contained two agglutinins, and I.S.4 apparently contained two agglutinins different from those of I.S.2 and 3. Corroboration of these results, it was felt, was necessary as it might well establish the existence of serological variations in *T. vaginalis*. As a preliminary check, it was decided to take two dissimilar sera and test a number of specimens of trichomonads against them. Through a laboratory mishap Antigens 1 and 2 died, and I.S. 3 and 4 consequently had to be used. Ten specimens of *T. vaginalis* (again from different and unselected patients) were tested against these sera. Four of the specimens were agglutinated by I.S.3 and six by I.S.4. No specimen was agglutinated at dilutions above 1 : 40 by both immune sera (Table VII).

TABLE VII
AGGLUTINATION RESULTS OF TEN SPECIMENS OF *T. VAGINALIS* AGAINST IMMUNE SERA 3 AND 4

No. of Specimens of <i>T. vaginalis</i>	Immune Serum		
	3	4	3 and 4
10	4	6	Nil

These results therefore substantiated the previous findings that I.S.3 and 4 were serologically distinct,

but "absorption tests" appeared essential to exclude the possibility of non-specific agglutination. Because of the accidental loss of Antigens 1 and 2, attempts were made to absorb I.S.1 with Antigen T.3, and the absorbed serum was then to be tested against Antigens 3 and 4.

Absorption Technique

A sample of I.S.1 which had previously been inactivated for 30 min. at 56°C., was set up in a 2-ml. volume. To this, 0.1 ml. of a suspension of washed, packed trichomonads (T.3) was added. Three additions were made at 30-min. intervals; after each addition the serum was incubated at 37°C. for 30 min. The serum was then centrifuged and separated from the organisms. The absorbed serum was then tested against Antigen 3 and 4. The result was significant in so far that no agglutination of Antigen 3 occurred, whereas Antigen 4 was agglutinated in all dilutions.

Further cross-absorption tests would appear essential before definite proof of serological variations can be claimed. This investigation is, therefore, as yet incomplete, but sufficient evidence has been produced to indicate that the existence of distinct strains of *T. vaginalis* is extremely likely. No attempt has been made to correlate these findings with clinical details. Such a correlation might or might not be found, but even if the serological findings were quite irrelevant to the clinical side of trichomoniasis it would present interesting epidemiological possibilities.

In conclusion it is pertinent to refer to intradermal tests carried out on the four immunized

rabbits. These were each given intradermal injections of the respective antigen (containing 2 million organisms per ml.) in 0.2-ml. doses. Control injections of medium were also given. No differences in reaction were noticeable. These results confirm previous reports of the negative value of intradermal tests in *T. vaginalis* infections.

Summary

Immune serum prepared against *T. vaginalis* showed the presence of agglutinating bodies.

Twenty specimens of *T. vaginalis* were tested against this immune serum: eighteen specimens were agglutinated and two were not agglutinated.

Four immune sera from different specimens of *T. vaginalis* were prepared: cross-agglutination tests indicated that two of the sera contained different agglutinins.

Of ten specimens of *T. vaginalis* tested against these two sera, six were agglutinated by one serum and four by the other. No specimen was agglutinated by both sera.

Confirmation of the different agglutinin content of the sera was provided by a cross-absorption test; it is felt that further cross-absorption tests should be carried out to support this finding.

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