Cultural and Serological Studies on *Haemophilus vaginalis*

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SUMMARY

When several strains of *Haemophilus vaginalis* were grown on Casman rabbit blood agar, individual morphological and cultural differences were noted between the Amies strains which formed pleomorphic and filamentous organisms and large, umbonate colonies, and the Dukes, Edmunds, King and U/L strains which were microscopically coccobacillary to bacillary, non-filamentous, and formed minute convex smooth colonies. Dukes, Edmunds, King and U/L strains required whole blood for maintenance while a whole blood derivative, e.g. peptic digest or Difco chocolate-yeastolate agar, was sufficient for the maintenance of the Amies strains. Serological studies by tube agglutination, direct, indirect and inhibition immunofluorescent methods showed that Dukes, Edmunds, King and U/L strains reacted in a homologous manner with *H. vaginalis* antisera nos. 317, 394 and 4984. Amies strains did not react with these antisera. However, Amies strains cross-reacted with *H. aegyptius* antiserum 180A, while the Dukes, Edmunds, King and U/L strains did not react with this antiserum.

INTRODUCTION

Several investigators (Leopold, 1953; Gardner & Dukes, 1954, 1955; Gardner, Dampeer & Dukes, 1957; Amies & Jones, 1957; Brewer, Halpern & Thomas, 1957; Edmunds, 1960*a*; Lapage, 1961; and others) have reported finding a small Gram-negative bacillus in the human female and male genito-urinary tract. These investigators used various media for the isolation and cultivation of this organism, named *Haemophilus vaginalis* by Gardner & Dukes (1955). Although most of these investigators used the criteria for identification established by Gardner & Dukes, several reports were at variance with the cultural behaviour and staining characteristics. Amies & Jones (1957) noted smooth-rough (S-R) colony forms of their strains, and their rough colonies contained more filamentous forms of the organism than did the smooth. No other investigators have reported S-R dissociation of *H. vaginalis*, nor have they reported filamentous forms. Variability in Gram reaction was observed by Brewer *et al.* (1957), Lutz & Burger (1957) and Edmunds (1960*a*). Dukes & Gardner (1961) reported Gram variability in 72 and 90 hr. cultures and associated this with the formation of volutin granules. Edmunds (1960*a*) related Gram variability to volutin granules which he believed to be formed by the fermentation of certain sugars, in particular glucose. Later he found (1960*b*) that haemolytic activity

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varied with the animal source of red blood cells. On his meat digest agar he obtained optimal haemolysis with human red blood cells whilst lysis of rabbit red blood cells was variable. Dukes & Gardner (1961) observed a green to brown discoloration type of haemolysis on Casman blood agar medium to be produced by most of the strains investigated. In view of these differences it was decided to make a comparative cultural study of representative strains and to determine whether serological studies might bear out the cultural differences. Strains of *H. aegyptius* and *H. influenzae* were also included in the study.

METHODS

Organisms used

Haemophilus vaginalis: strains 317, 394, 513 and 594 from Dr C. D. Dukes; strains H 36, H 76, H 2030 from Dr C. R. Amies; strain T 145 from Dr P. N. Edmunds; strain 4984 (King strain) from Miss E. O. King; strains 842 and 867 (U/L strains) from University of Louisville.

Haemophilus aegyptius: strains 180 A and 181 A from Dr M. Pittman.

Haemophilus influenzae: ATCC strains 8412, 9006, 9007, 9008 and 9334 from the American Type Culture Collection.

Media. Casman (1947) broth and agar (Difco) + 5 % fresh defibrinated rabbit blood. Chocolate-yeastolate (Difco) agar (5 % defibrinated rabbit blood and 1 % yeastolate added to heart infusion agar base). Fildes peptic digest agar (1920) with defibrinated rabbit blood and Casman agar or heart infusion agar.

Incubation was at 37° in a candle jar.

Staining. Smears of *Haemophilus vaginalis* cultured for up to 5 days in Casman rabbit blood agar were prepared, air dried and heat-fixed. The smears were stained by the potassium mercuric iodide modification of the Gram method, by Giemsa and by basic fuchsin dilute solution. Strains of a vaginal diphtheroid and of *Staphylococcus aureus* were used as Gram-positive controls.

Antiserum preparation. Antisera for *Haemophilus vaginalis* strains 317, 394 and 4984, and for *H. aegyptius* strain 180 A were prepared by inoculating live organisms ($3-4 \times 10^6$ bacteria/ml. sterile saline) intravenously, and formalin-killed organisms mixed with Freund adjuvant (Difco) subcutaneously, into the heel pad of normal, healthy New Zealand male rabbits (4-6 lb.) at various times during 24 days. Antisera were obtained one week following the last inoculation by sterile cardiac puncture, and stored at -60° for subsequent use. Antisera for *H. vaginalis* strains 317 and 4984 and for *H. aegyptius* strain 180 A were used to prepare fluorescein-conjugated globulins by the method of Cherry, Goldman, Carski & Moody (1960). Human liver powder and bovine red bone marrow were used for the adsorption of the globulin conjugates to decrease non-specific fluorescence. Immunofluorescent reactions were carried out by direct, indirect and inhibition methods on air-dried heat-fixed bacterial smears. The fluorescence equipment used in these studies had an HB-200 mercury vapour bulb as a light source, with two Corning 5840 exciter filters in one case, and a BG12 exciter filter in the other.

Agglutination tests. Tube agglutination tests (Evans, 1957) were done with live bacteria of various species and strains derived from 18 hr. cultures. The diluent was Hanks balanced salt solution containing 0.1 % bovine serum albumin. The tubes

were incubated in a 37° water bath for 3 hr. and examined macroscopically and microscopically for agglutination. The tubes were then held at 4° for 18 hr., and again examined for agglutination.

RESULTS

Morphology and staining reactions

Smears made from 18 hr. Casman rabbit blood agar cultures showed a bacillus ranging from coccobacillary to bacillary forms for the Dukes, King, Edmunds and U/L strains of *Haemophilus vaginalis*; the three Amies strains when grown on Casman medium showed extreme pleomorphism, with many filamentous forms. The

Table 1. *Growth of various strains of Haemophilus vaginalis*

Strain	Casman agar	Casman rabbit blood agar	Casman rabbit blood broth	Fildes peptic digest agar	Chocolate yeastolate agar
Dukes 513	—	+	+	—	±
Dukes 594	—	+	+	—	±
Dukes 394	—	+	+	—	±
Edmunds T145	—	+	+	—	±
King 4984	—	+	+	—	±
U/L 867	—	+	+	—	±
U/L 842	—	+	+	—	±
Amies H76	—	+	±	+	+
Amies H36	—	+	±	+	+
Amies H2030	—	+	±	+	+
Pittman 180A*	—	+	—	+	+

+, Growth; ±, sparse growth (could not be subcultured); —, no growth.

* a strain of *H. aegyptius*

Table 2. *Tube agglutination reaction*

Antigen	Reciprocal of agglutination titre with antisera							
	<i>H. vaginalis</i> 317		<i>H. vaginalis</i> 4984		<i>H. vaginalis</i> 394		<i>H. aegyptius</i> 180A	
	3 hr.	18 hr.	3 hr.	18 hr.	3 hr.	18 hr.	3 hr.	18 hr.
	3 hr.	18 hr.	3 hr.	18 hr.	3 hr.	18 hr.	3 hr.	18 hr.
<i>H. vaginalis</i> 317 (Dukes)	5120	5120	1280	1280	640	640	10	10
<i>H. vaginalis</i> 513 (Dukes)	640	640	320	640	320	320	0	0
<i>H. vaginalis</i> 394 (Dukes)	640	640	320	320	2560	2560	10	10
<i>H. vaginalis</i> 594 (Dukes)	320	320	640	640	640	640	0	0
<i>H. vaginalis</i> 4984 (King)	640	640	5120	5120	320	320	0	0
<i>H. vaginalis</i> T145 (Edmunds)	320	320	640	1280	640	1280	0	0
<i>H. vaginalis</i> 842 (U/L)	640	640	320	320	320	320	0	0
<i>H. vaginalis</i> 867 (U/L)	160	320	1280	1280	320	640	0	0
<i>H. vaginalis</i> H2030 (Amies)	10	10	10	10	10	10	40	80
<i>H. vaginalis</i> H76 (Amies)	20	10	10	0	0	0	40	80
<i>H. aegyptius</i> 180A (Pittman)	0	10	10	10	0	0	2560	2560
<i>H. aegyptius</i> 181A (Pittman)	0	0	0	0	0	0	1280	1280
<i>H. influenzae</i> 9008 (ATCC)	0	0	0	0	0	0	0	0
<i>H. influenzae</i> 8412 (ATCC)	0	0	0	0	0	0	0	0
<i>H. influenzae</i> 9006 (ATCC)	0	0	0	0	0	0	0	0
<i>H. influenzae</i> 9334 (ATCC)	0	0	0	0	0	0	0	0
<i>H. influenzae</i> 9007 (ATCC)	0	0	0	0	0	0	0	0

Table 3. *Inhibition immunofluorescent reactions*

Antigen	Non-fluorescent <i>H. vaginalis</i> antiserum 317 dilutions									Non-fluorescent <i>H. vaginalis</i> antiserum 4984 dilutions									Normal rabbit serum controls					
	0	10	20	40	80	160	320	640	0	10	20	40	80	160	320	640								
<i>H. vaginalis</i> 4984 (King)	+	+	+	±	-	-	-	-	+	+	+	+	+	+	±	-								
<i>H. vaginalis</i> 317 (Dukes)	+	+	+	+	+	±	±	-	+	+	+	±	-	-	-	-								
<i>H. vaginalis</i> 394 (Dukes)	+	+	+	±	-	-	-	-	+	+	+	±	±	-	-	-								
<i>H. vaginalis</i> 842 (U/L)	+	+	+	±	-	-	-	-	+	+	+	+	±	-	-	-								
<i>H. vaginalis</i> T 145 (Edmunds)	+	+	+	+	±	-	-	-	+	+	+	+	+	±	±	-								
<i>H. aegyptius</i> 180 A (Pittman)	±	-	.	.	.	.	.	.	±	-	.	.	.	.	.	.								
Antigen	Non-fluorescent <i>H. vaginalis</i> antiserum 394*									Non-fluorescent <i>H. aegyptius</i> antiserum 180A dilutions									Normal rabbit serum controls					
	0	10	20	40	80	160	320	640	0	10	20	40	80	160	320	640								
<i>H. vaginalis</i> 4984 (King)	+	+	+	±	-	-	-	-	-	.	.	.	.	.	.	.								
<i>H. vaginalis</i> 317 (Dukes)	+	+	+	±	±	-	-	-	-	.	.	.	.	.	.	.								
<i>H. vaginalis</i> 394 (Dukes)	+	+	+	+	+	+	±	-	-	.	.	.	.	.	.	.								
<i>H. vaginalis</i> 842 (U/L)	+	+	+	±	-	-	-	-	-	.	.	.	.	.	.	.								
<i>H. vaginalis</i> T 145 (Edmunds)	+	+	+	±	-	-	-	-	-	.	.	.	.	.	.	.								
<i>H. aegyptius</i> 180 A (Pittman)	-	.	.	.	.	.	.	.	+	+	+	+	+	+	+	-								

* Fluorescein-tagged *H. vaginalis* 4984 antiserum was used in the inhibition reactions with non-fluorescent antiserum 394. +, complete inhibition (no fluorescence); ±, incomplete inhibition (weak fluorescence); -, no inhibition (fluorescence).

size of basic fuchsin-stained organisms ranged from $0.4-0.6 \mu \times 1.0-2.0 \mu$ for the Dukes, King, Edmunds and U/L strains, whereas the Amies strains tended to be slightly longer, $2.0-3.5 \mu$.

Organisms from such cultures were Gram-negative. Gram variability was noted occasionally with some strains (Edmunds, King) in smears made from 48 and 72 hr. In Casman rabbit blood broth cultures, such Gram variability was not observed with the Amies (H2030), Dukes or U/L (842) strains. Bipolar staining was observed in some Giemsa stained organisms from Casman rabbit blood agar cultures of Dukes, Edmunds, King and U/L strains. Two of the Amies strains (H36, H76) did not show bipolar staining, while strain H2030 showed a few bipolar organisms. The Amies strains showed filamentous forms on Casman rabbit blood agar.

Table 4. *Direct immunofluorescent reaction*

Organism and source	Fluorescent antiserum		
	<i>H. vaginalis</i> 317	<i>H. vaginalis</i> 4984	<i>H. aegyptius</i> 180A
<i>H. vaginalis</i> 317 (Dukes)	+	+	—
<i>H. vaginalis</i> 394 (Dukes)	+	+	—
<i>H. vaginalis</i> 4984 (King)	+	+	—
<i>H. vaginalis</i> T104 (Edmunds)	+	+	—
<i>H. vaginalis</i> T145 (Edmunds)	+	+	—
<i>H. vaginalis</i> 680 (U/L)	+	+	—
<i>H. vaginalis</i> 842 (U/L)	+	+	—
<i>H. vaginalis</i> 867 (U/L)	+	+	—
<i>H. vaginalis</i> H36 (Amies)	—	—	+
<i>H. vaginalis</i> H76 (Amies)	—	—	±
<i>H. vaginalis</i> H2030 (Amies)	—	—	+
<i>H. aegyptius</i> 180A (Pittman)	—	—	+
<i>H. aegyptius</i> 181A (Pittman)	—	—	+
<i>H. influenzae</i> 9006 (ATCC)	—	—	+
<i>H. influenzae</i> 9007 (ATCC)	—	—	+
<i>H. influenzae</i> 9008 (ATCC)	—	—	+
<i>H. influenzae</i> 9334 (ATCC)	—	—	+
<i>H. influenzae</i> 8142 (ATCC)	—	—	+

+, fluorescence; ±, weak fluorescence; —, no fluorescence.

Colonial morphology. The colonial morphology of the various *Haemophilus vaginalis* strains on Casman rabbit blood agar were studied after 24 hr. and 48 hr. incubation in a candle jar. The Dukes, Edmunds, King and U/L strains formed colonies like those described by Dukes & Gardner (1961). The Amies strains, however, were umbonate, entire, translucent and slightly granular, varying in size from 0.37–0.97 mm. in diameter. They were similar in this respect to *H. aegyptius* strain 180A.

Haemolytic activity on rabbit red cells in Casman medium showed a wide range of variability from no reaction to slight green colour, and to greenish-brown after 24 hr. for all strains of *H. vaginalis*; a greenish area with haemolysis was most frequently observed, however. No haemolysis was produced by *H. aegyptius*.

Maintenance on various media. The maintenance of *Haemophilus vaginalis* strains and one strain of *H. aegyptius* was studied. The results of these studies are summarized in Table 1 from which it is apparent that whole blood or perhaps serum is necessary for the maintenance of Dukes, Edmunds, King and U/L strains.

Table 5 (cont.)

Antigen	Non-fluorescent <i>H. vaginalis</i> antiserum 394 dilutions										Non-fluorescent <i>H. aegyptius</i> antiserum 180 A dilutions										Normal rabbit serum control				
	0	10	20	40	80	160	320	640	0	10	20	40	80	160	320	640	317	4984	394	180 A					
<i>H. vaginalis</i> 4984 (King)	4	2	1	tr	—	.	.	.	—	.	.	.	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> 317 (Dukes)	4	2	1	1	tr	—	.	.	—	.	.	.	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> 394 (Dukes)	4	4	3	3	2	1	—	.	—	.	.	.	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> 842 (U/L)	4	2	1	—	.	.	.	.	—	.	.	.	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> T145 (Edmunds)	4	3	2	1	—	.	.	.	—	.	.	.	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> H 2030 (Amies)	—	.	.	.	.	.	.	.	3	2	1	—	.	.	.	.	—	—	—	—					
<i>H. vaginalis</i> H76 (Amies)	—	.	.	.	.	.	.	.	3	2	1	1	—	.	.	.	—	—	—	—					
<i>H. vaginalis</i> H 36 (Amies)	—	.	.	.	.	.	.	.	3	2	1	—	.	.	.	.	—	—	—	—					
<i>H. aegyptius</i> 180 A (Pittman)	—	.	.	.	.	.	.	.	4	4	3	3	2	tr	—	.	—	—	—	—					

4 to 1, degree of fluorescence observed (4 = maximum); tr, weak fluorescence observed; —, no fluorescence observed.

Agglutination tests. Results of tube agglutination reactions at 3 hr. and 18 hr. incubation are shown in Table 2. The highest agglutination titres were obtained in homologous antigen and antiserum reactions. Significantly high titres were obtained, however, in some of the heterologous reactions with *Haemophilus vaginalis*. The titres of the Amies strains (H76 and H2030) with the antisera of *H. vaginalis* 317, 4984 and 394 are not significant, although the titres for the *H. aegyptius* 180A antiserum may be significant. Spontaneous agglutination of *H. vaginalis* H36 (Amies) was not avoided even when 0.1 % bovine serum albumin modified Hanks balanced salt solution was used; therefore, this strain was not included in the tube agglutination studies.

Immunofluorescent reactions. Specificity of the fluorescent *Haemophilus vaginalis* (317 and 4984) and *H. aegyptius* (180A) antisera were determined by the inhibition reactions (see Table 3). Inhibition by non-fluorescent antiserum was greatest in the homologous antigen-globulin reaction. However, the non-fluorescent *H. vaginalis* antiserum reacted with heterologous *H. vaginalis* strains, resulting in inhibition of the fluorescent reaction. No significant inhibition was noted when non-fluorescent *H. vaginalis* 317 and 4984 antisera were mixed with *H. aegyptius* 180A antigen.

The results of the cross-reaction studies by the direct method with species of *Haemophilus* are shown in Table 4. *H. vaginalis* fluorescent globulins 317 and 4984 reacted with homologous and heterologous *H. vaginalis* strains Dukes, Edmunds, King and U/L, but not with the Amies strains (H36, H76, H2030). These Amies strains did show a reaction with *H. aegyptius* fluorescent globulin 180A, as did *H. influenzae* strains and the *H. aegyptius* strains. Strains of *H. vaginalis* (Dukes, King and U/L) other than those listed in Table 4 were also studied; all of these showed a fluorescent reaction of varying intensity with *H. vaginalis* fluorescent globulins 317 and 4984. No fluorescent reaction was observed with these strains when *H. aegyptius* fluorescent globulin 180A was used. Indirect immunofluorescent reactions were made to further substantiate the specificity of direct and inhibition tests. The results are in Table 5.

DISCUSSION

The morphological and colonial characteristics of the Edmunds, King and U/L strains of *Haemophilus vaginalis* grown on Casman rabbit blood agar were consistent with the report of Dukes & Gardner (1961). The characteristics of the Amies strains, however, differed on this medium, where they formed larger and umbonate colonies. Gram variability was noted in only two strains (Edmunds and King), and was not a consistent phenomenon in these two strains. In agreement with Dukes & Gardner (1961), bipolar staining was observed frequently in Dukes, Edmunds, King and U/L strains of *H. vaginalis*, but was observed in only one Amies strain (H2030).

All investigators with the exception of Amies & Jones (1957) have noted that whole blood from one or another animal was a cultural requirement for strains of *Haemophilus vaginalis*. In the present work it was noted that Amies strains could be easily maintained on peptic digest agar and chocolate-yeastolate agar, both of which contain factors derived from whole blood. However, the Dukes, Edmunds, King and U/L strains could not be maintained on these media, thus indicating that

some factor in whole blood or serum was needed by the latter strains, and was not provided by the whole blood derivatives.

Lapage (1961) used Casman agar in addition to other media in comparing his isolates with strains of *Haemophilus vaginalis* from Drs C. R. Amies and C. D. Dukes. Lapage established two cultural groups: (1) *H. influenzae*-like, which included the Amies strains of *H. vaginalis*; (2) *H. vaginalis*-like, which included a Dukes strain of this organism. On Casman rabbit blood agar a similar grouping was found in the present work, the Amies strains being similar to *H. aegyptius* in maintenance and morphology, while the Edmunds, King and U/L strains were identical with the strains of *H. vaginalis* obtained from C. D. Dukes.

In the present work, the majority of strains produced a haemolysis as described by Dukes & Gardner (1961); however, an occasional strain produced no haemolysis or produced an inner zone of β -haemolysis, in agreement with Edmunds (1960*a*). Because of these varied results, we believe that haemolysis is of little value in the identification of *Haemophilus vaginalis* colonies on Casman rabbit blood agar.

Colonies of *Lactobacillus*, *Streptococcus* and diphtheroids may be easily confused with *Haemophilus vaginalis* colonies macroscopically; it is therefore important to examine the minute colonies microscopically for granularity and irregular shape.

Haemophilus vaginalis is an extremely fastidious organism, and maintenance of stock cultures over long periods of time was difficult. Best results were obtained when the Dukes, Edmunds, King and U/L strains were transferred daily in Casman rabbit blood broth; other media suggested for the maintenance of the genus *Haemophilus* failed to support their growth. Casman rabbit blood broth and agar were used as the medium of choice for *H. vaginalis*. Amies strains differed from the above strains in that they could not be maintained in Casman rabbit blood broth; they were maintained successfully by transferring at 3- to 5-day intervals on chocolate-yeastolate agar or on Casman rabbit blood agar.

Dukes & Gardner (1961) stated that the X and V factors are stimulatory accessory growth factors for *Haemophilus vaginalis* in the presence of partially digested protein, rabbit serum and certain amino acids. We found it impossible to supply the nutritional requirements of these strains by using a peptic digest prepared from whole rabbit blood or by using yeastolate with whole rabbit blood that had been denatured by heat. Thus, some factor or factors other than the X and V of whole blood are necessary for these strains. On the other hand, X and/or V factor requirements of Amies strains could be supplied by derivatives of whole rabbit blood such as a peptic digest of such blood or chocolate-yeastolate agar.

Agglutination reactions. The addition of 0.1 % bovine serum albumin to a modified balanced salt solution (e.g. Hanks) eliminated spontaneous agglutination in tube reactions with all strains of *Haemophilus vaginalis* except Amies strain H36. No cross-reactions occurred with *H. influenzae*, *H. aegyptius* and Amies strains of *H. vaginalis* and the three *H. vaginalis* antisera (317, 394 and 4984). Homologous and heterologous strains of *H. vaginalis* (Edmunds, Dukes, King and U/L) were agglutinated by the three *H. vaginalis* antisera. In this investigation as in the investigation of Dukes & Gardner (1961) the homologous strains produced high titres, whereas the heterologous strains produced lower titres.

Amies & Jones (1957) did not observe cross-reactions with antisera for their strains of *Haemophilus vaginalis* and strains of *H. influenzae* and *Bordetella pertussis*

antigens by the slide-agglutination test; however, they did not include *H. aegyptius* in their investigation. *H. aegyptius* antiserum agglutinated Amies strains of *H. vaginalis* at titres of 1/80 and did not agglutinate Dukes, Edmunds, King and U/L strains of *H. vaginalis* or *H. influenzae*. Pittman & Davis (1950) stated that cross-reactions with heterologous strains of *H. aegyptius* and *H. aegyptius* antisera at titres of 1/40 were significant. In view of the cross-reactions observed with Amies strains of *H. vaginalis* and *H. aegyptius* antiserum in the present work, it appears that these strains are more closely related to *H. aegyptius* than to *H. vaginalis*.

Immunofluorescent reactions correlated with the agglutination reactions. Cross-reactions did not occur in reactions with *Haemophilus vaginalis* antiserum (317 and 4984) and antigens (whole bacteria) of *H. influenzae*, *H. aegyptius*, and Amies strains of *H. vaginalis*. Reactions did occur, however, with *H. vaginalis* antiserum (317 and 4984) and any of the Dukes, Edmunds, King and U/L strains of *H. vaginalis*. On the basis of these observations, it appears there are no common antigens shared by *H. vaginalis*, *H. influenzae* and *H. aegyptius* on the one hand and Amies strains of *H. vaginalis* on the other. Cross-reactions did occur with *H. aegyptius* fluorescent globulin and Amies strains of *H. vaginalis* and *H. influenzae* by the direct immunofluorescent method, thus indicating common antigens present in the three species. Further evidence of Amies strains of *H. vaginalis* sharing common antigens with *H. aegyptius* was provided by the indirect immunofluorescent method.

The inhibition and indirect immunofluorescent titres differed from the tube-agglutination titres with the same serum by a twofold or greater dilution factor. Such variation has been noted previously by Goldman (1957) during his investigation of *Toxoplasma*, and by Biegeleisen, Bradshaw & Moody (1962) in their investigations of *Brucella* anti-bodies in human serum. Although such titres varied, the immunofluorescent reactions are considered to be highly specific.

The specificity of the fluorescent *Haemophilus vaginalis* (317 and 4984) globulin was determined by inhibition and direct immunofluorescent methods. Cross-reactions which occurred with other species of *Haemophilus* and undiluted fluorescent globulin were avoided by diluting the fluorescent antisera 1/12 in the direct immunofluorescent method.

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