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ROLE OF DIVALENT CATIONS, pH, CYTOSKELETON COMPONENTS AND SURFACE CHARGE ON THE ADHESION OF *TRICHOMONAS VAGINALIS* TO A POLYSTYRENE SUBSTRATE

FERNANDO COSTA E SILVA FILHO*, CEZAR ANTONIO ELIAS** & WANDERLEY DE SOUZA**

* Instituto Oswaldo Cruz, Departamento de Protozoologia, Caixa Postal 926, 20001 Rio de Janeiro
** Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941 Rio de Janeiro, RJ, Brasil

The process of adhesion of three different strains of Trichomonas vaginalis *to a polystyrene substrate was analysed. The process of adhesion was dependent on the time of incubation and the pH of the phosphate-buffered solution (PBS) in which the parasites were suspended. The highest indices of adhesion were observed after an incubation time of 60 min at pH 6.6. The adhesion index increased when the parasites were incubated in the presence of culture media or when Ca^{++} or Mg^{++} was added to the PBS solution, whereas cytochalasin B, trypsin or neuraminidase reduced adhesion. Incubation of the parasites in the presence of poly-L-lysine facilitated the process of adhesion. Incubation of the parasites or polystyrene beads in the presence of poly-L-lysine led to important changes in their surface charge.*

Key words: *Trichomonas vaginalis* – cell adhesion – cytoskeletal components – surface charge

Adhesion of some parasitic protozoa to the surface of host cells plays an important role in pathogenicity. *Trichomonas vaginalis* which is found in the human urogenital tract may interact with epithelial cells through its surface and exerts its pathogenic action outside the host cells (review in Honigberg, 1978, 1979; Brasseur & Savel, 1982). *T. vaginalis* adheres to the surface of various types of solid substrates (Lumsden et al., 1966) by a process in which surface glycoproteins (Cappuccinelli et al., 1975) and cytoskeletal components of the parasite seem to be involved (Cappuccinelli & Varesio, 1975).

Analysis of the binding of some lectins to the cell surface (Warton & Honigberg, 1983) and the determination of the surface charge (Silva Filho et al., 1986) of 3 strains of *T. vaginalis* suggest that differences in cell surface properties exist among strains with different grades of pathogenicity. It has been well established that the surface properties of the cells play a fundamental role on their ability to adhere to different types of inert and biological substrata. In the present work we analysed the process of adhesion of 3 strains of *T. vaginalis* previously characterized by cell electrophoresis (Silva Filho et al., 1986) to a polystyrene substrate. We studied the influence of (a) cytoskeletal components of the parasite, (b) surface molecules sensitive to trypsin or neuraminidase treatment, (c) components of the incubation medium such as serum, Ca^{++} and Mg^{++} and, (d) pH of the incubation medium.

MATERIALS AND METHODS

Strains of T. vaginalis – The Jt, RT and IOC strains used in this work were characterized previously (Silva Filho et al., 1986). All strains were maintained in TYM medium (Diamond, 1957) at 37°C or at room temperature, by passages at 3 days intervals. For our experiments the cells were grown for 36-40h at 37°C in TYM (Diamond, 1957) medium without agar, and supplemented with 10% bovine serum using GasPak anaerobic jars. The parasites were collected by centrifugation (1500 rpm for 15 min at 4°C), washed in phosphate-buffered saline (PBS), pH 6.6 and resuspended in PBS at a concentration of 1.3×10^5 cells/ml. Cell viability was checked using the erythrosin B exclusion test (Phillips & Terryberry, 1957).

Reagents – Cytochalasin B, dissolved as a 1% (w/v) stock solution in dimethylsulfoxide, colchicine, trypsin (type III), neuraminidase from *Clostridium perfrigens* (type X), poly-L-lysine (molecular weight: 70000), ascorbic acid, and cysteine were purchased from Sigma.

Enzymatic treatment – Whole parasites were incubated with 0.2 U/ml neuraminidase or 500 μg/ml trypsin, as described previously (Silva Filho et al., 1982). After enzymatic treatment the cells were washed twice in PBS and used in adhesion assays.

Measurement of cell adhesion – Cell adhesion to a polystyrene substrate was measured

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by incubating 2 x 10^5 parasites, suspended in 2 ml of medium containing test substances at 37°C for 30 to 120 min in 35 x 10 mm culture dishes. After incubation the dishes were washed twice with PBS and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. A transparent grid with squares of 4 mm^2 was placed below the culture dishes and then observed with an inverted microscope. The number of cells in each square was determined in at least four randomly selected squares. In all experiments the adhesion values obtained for cells which were incubated in the presence of PBS were considered as 100%. The data shown in the Tables represent the results obtained in 2 to 5 experiments. In some experiments, ions (Ca^{++}, Mg^{++}) or substances (colchicine, cytochalasin B, dimethylsulphoxide), were added to the PBS solution in which the parasites were suspended. In other experiments the parasites were incubated for 30 min at room temperature in the presence of 0.01, 0.1, 1.0 or 2.0% poly-L-lysine before the adhesion assay. Statistical comparisons of differents media and treatments were performed using the Student's "t" test.

Cell electrophoresis – The electrophoretic mobility of control *T. vaginalis* as well as parasites and polystyrene beads (mean diameter, 4.43 μm) previously incubated for 30 min at room temperature in the presence of poly-l-lysine (0.01, 0.1, 1.0 or 2.0%) was determined as described previously (Silva Filho et al., 1982).

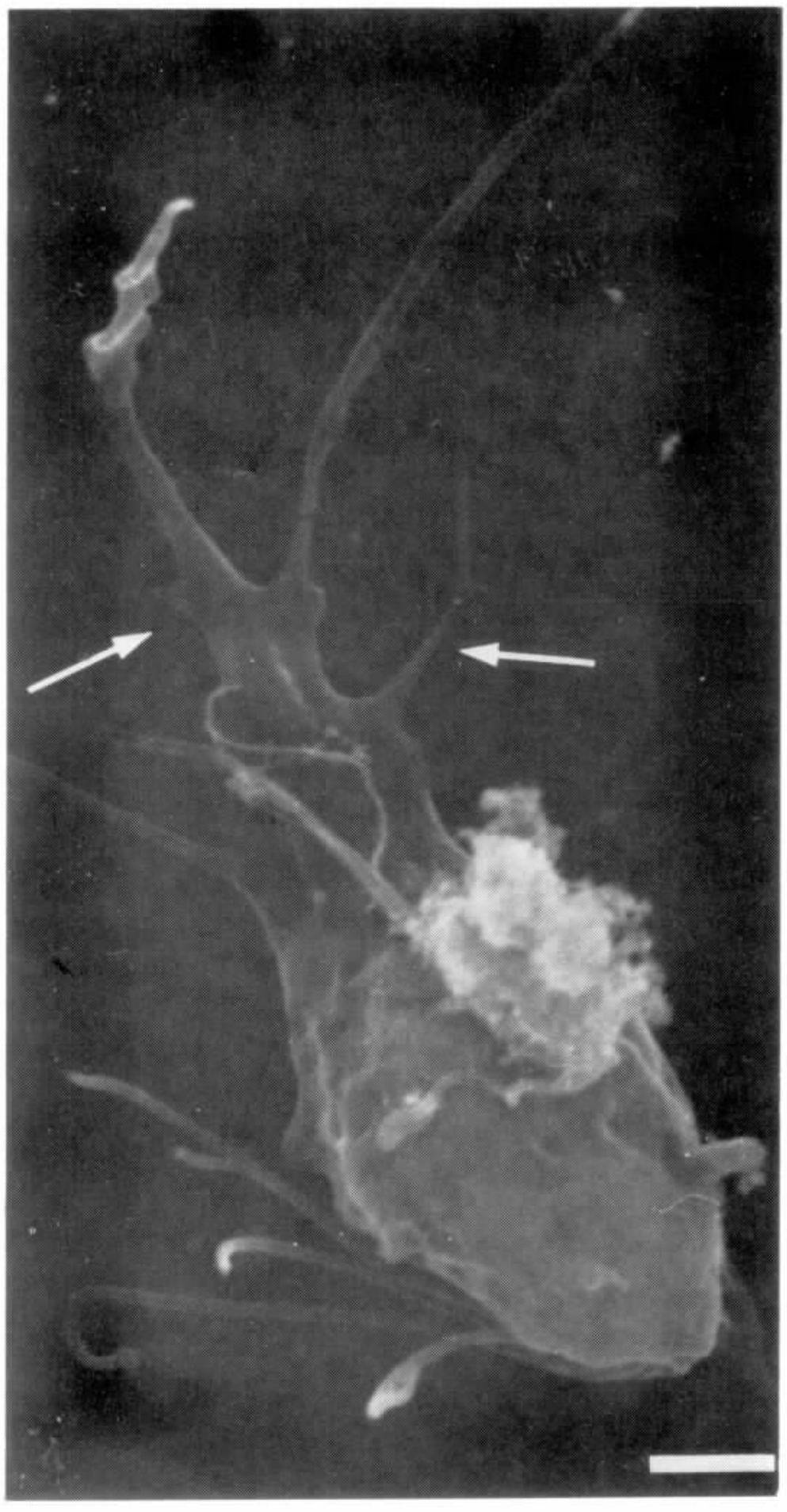
RESULTS

Morphology – Observations made with the light microscope showed that many parasites randomly adhered to the surface of polystyrene dishes and did not detach if the dishes were washed several times with PBS or incubated at 0°C. Scanning electron micrographs of the adhered cells showed the presence of filopodium-like surface projections mainly located in the posterior region of the parasite (Fig. 1) Usually these projections are not seen in free cells.

Measurement of parasite's adhesion – Table I shows the effect of the time of incubation of parasites suspended in PBS on their adhesion to the polystyrene substrate. The adhesion was highest after 60 min of incubation. After longer periods of incubation some cells expontaneously detached from the substrate. The three strains of *T. vaginalis* analysed adhered to the substrate to about the same extent. In view of these results an incubation time of 60 min was selected for the experiments described below.

As shown in Table II the pH of the PBS solution in which the parasites were suspended interfered with attachment of the parasites to the substrate. It was low at pH 4.3 as well as at pH 7.5. The influence of the pH of the medium in which the cells were suspended on their attachment to the substrate was more intense for parasites of the IOC strain than for the other strains analysed (see the adhesion indexes at pH 7.5). Based on the data obtained (Table II) pH 6.6 was selected for all experiments described below.

The nature of the medium in which the parasites were suspended interfered with their adhesion to the substrate. It was about 30% higher when the parasites were suspended in fresh culture medium than in PBS alone (data not shown).



Scanning electron micrograph showing a parasite (Jt strain) adhered to the polystyrene substrate. Filopodium-like surface projections are seen in the posterior region of the cell (large arrows). The short arrows indicate the flagella; bar = 1 um.

TABLE I

Effect of the time of incubation of three different strains of *Trichomonas vaginalis* on their adhesion to a polystyrene substrate*

Time of incubation (min)	Number of adhered parasites (x 10^4) per mm^2					
	Jt	%	Rt	%	IOC	%
30	2.95	14.8	2.72	13.6	3.15	15.8
60	5.80	29.0	5.12	25.6	5.45	27.3
90	4.95	24.8	4.60	21.3	5.07	25.4
120	3.65	18.3	4.22	21.1	3.77	18.9
180	3.47	17.4	2.42	12.1	2.85	14.3

* The parasites were suspended in a PBS solution at pH 6.6.
The data are from one representative experiment.

TABLE II

Effect of the pH of the phosphate buffered saline solution (PBS) in which parasites were suspended on their adhesion to the polystylene substrate

pH	Number of adhered *T. vaganalis* ± SD *x $10^4/mm^2$ Strains		
	Jt	Rt	IOC
4.3	5.65 ± 2.95	4.27 ± 1.97	3.57 ± 2.00
5.5	7.85 ± 1.50	5.35 ± 1.6	6.07 ± 1.60
6.6	7.67 ± 1.72	7.04 ± 1.27	7.22 ± 2.52
7.5	5.27 ± 0. 1	3.85 ± 0.17	2.15 ± 1.52

For all strains 20 x 10^4 parasites/mm^2 were poured into plastic dishes.
*Standard deviation

The data from Table III show that the addition of 1 mM of Ca^{++} or Mg^{++} to the PBS solution in which the parasites were suspended increased their ability to adhere to the polystyrene substrate. Lower concentrations of these ions (10^{-6} M) did not interfere with the adhesion index. The effect of Ca^{++} was seen only in parasites of the Jt strain. The effect of Ca^{++} and Mg^{++} was not observed if EDTA was added to the medium (data not shown).

Addition of colchicine to the medium in which the parasites were suspended slightly inhibited the adhesion of parasites of the Rt strain (Table III) but did not interfere with the adhesion of parasites of the other two strains (not shown). Cytochalasin B, however, significantly inhibited the adhesion of parasites of the theree strains to the substrate. Dimethylsulphoxide, at the concentration found in the solution of cytochalasin B, did not interfere with the attachment of the parasite to the substrate (Table III).

Incubation of the parasites in the presence of trypsin or neuraminidase before the interaction with the substrate significantly inhibited their adhesion to the polystyrene substrate. This effect was observed in the three strains of *T. vaginalis* used (Table IV).

Incubation of the parasites for 30 min at 37°C in the presence of poly-L-lysine, followed by washing and ressuspension in PBS, when used at a concentration of 0.1%, significantly increased the adhesion of parasites of the Jt strain and slightly increased the adhesion of parasites of the Rt and IOC strains to the substrate. No effect was observed when lower concentration of poly-L-lysine were used, except with the Jt strain (Table IV).

Cell electrophoresis – Table V shows the mean cellular electrophoretic mobility of control and poly-L-lysine-treated polystyrene beads and parasites. Polystyrene beads, with a mean diameter of 4.43 μm, had a net negative surface charge. After incubation in the presence of poly-l-lysine they became positive. Incubation of parasites of the Jt strain in the presence of poly-l-lysine decreased their negative surface charge. This effect was dependent on the con-

TABLE III

Effect of divalent cations and cytoskeleton-disrupting drugs on the adhesion of *Trichomonas vaginalis* to polystyrene substrata

Treatment	Adhesion rates (mean value ± SD*) *T. vaginalis* strains	
	Jt	Rt
none	100%	100%
Ca^{++} (10^{-3}M)	144.6 ± 12.1% ($p < 0.001$)**	108.2 ± 15.3% (n.s)***
Mg^{++} (10^{-3}M)	140.8 ± 20.3% ($p < 0.001$)	128.1 ± 12.3% ($p < 0.01$)
Colchicine (1 μg/ml))	103.3 ± 17.5% (n.s.)	78.3 ± 15.8% ($p < 0.001$)
Cytochalasin B (10 μg/ml)	59.2 ± 11.7% ($p < 0.001$)	65.0 ± 4.2% ($p < 0.001$)
Dimethylsulfoxide (0.1%)	95.0 ± 15.0% (n.s.)	89.2 ± 11.7% (n.s.)

* Standard deviations

** Values between parenthesis are indicating the results of "t" test.

*** n.s., not significant.

TABLE IV

Adhesion of neuraminidase-treated, trypsin-treated, poly-L-lysine (PL) treated and untreated *T. vaginalis* on polystyrene substrate

Treatments	Adhesion rates ± SD* *T. vaginalis strains*		
	Jt	Rt	IOC
nome	100	100	100
trypsin	61.8 ± 9.7% ($p < 0.001$) *	48.2 ± 8.0% ($p < 0.001$)	56.2 ± 21.7% ($p < 0.001$)
neuraminidase	45.0 ± 2.1% ($p < 0.001$)	53.8 ± 7.2% ($p < 0.001$)	53.6 ± 2.8% ($p < 0.001$)
10^{-1}M (PL)	134.8 ± 11.2% ($p < 0.001$)	111.5 ± 10.6% (n.s.)	114.1 ± 14.5% (n.s.)
10^{-2}M (PL)	102.7 ± 7.3% (n.s.)	98.4 ± 17.5% (n.s.)	104.3 ± 2.2% (n.s.)
10^{-3}M (PL)	136.4 ± 18.3% ($p < 0.001$)	98.1 ± 8.2% (n.s.)	96.7 ± 10.8% (n.s.)

* Standard deviation

** Values between parenthesis are indicating the results of "t" test. n.s., not significant.

TABLE V

Effect of incubation of polystyrene latex beads and *Trichomonas vaginalis* in the presence of poly-L-lysine on the mean cellular electrophoretic mobility (EPM) and the Zeta Potencial [1,2].

System	EPM ± SD* ($\mu m.s^{-1}.V^{-1}.cm$)	Zeta potential (mV)	Percent change zeta-potential
Beads	−0.778 ± 0.012	−9.99	–
Beads + 0.1% PL [3]	+1.022 ± 0.008	+12.87	−228
Beads + 1% PL	+0.987 ± 0.010	+12.68	−226
T. viginalis	−1.015 ± 0.010	13.04	–
T. viginalis + 0.1% PL	−0.021 ± 0.015	− 2.58	− 80
T. viginalis + 1% PL	+0.156 ± 0.022	+ 2.00	−115
T. vaginalis + 2% PL	+0.188 ± 0.025	+ 2.41	−118

1. The latex beads has a mean diameter of 4.43 μm
2. The data were obtained with the Jt strain of *T. vaginalis*
3. PL, poly-L-lysine

centration of poly-l-lysine used. At high concentrations (1 or 2%) the surface of the cells became positive and they migrated towards the negative electrode (Table V).

DISCUSSION

Our observations confirm those reported previously showing that *T. vaginalis* attaches to some non-biological substrates (Lumsden et al., 1966). The three different strains of *T. vaginalis* tested by us have different levels of pathogenicity, as evaluated by their generation time in culture and their ability to induce abcesses in mice (Silva Filho et al., 1986), but they adhere to the polystyrene substrate to about the same extent. However, differences between them were observed when the effect of Ca^{++}, Mg^{++} and poly-l-lysine on their attachment to the substrate was tested.

Our results indicate that the pH of the PBS solution in which the parasites are suspended interfered with their attachment to the substrate. Studies carried out with other cell types have shown that cell adhesion increases when the pH is lowered, possibly as consequence of reduction in the ionization of carboxyl and other anions (review in Curtis, 1966). In the case of *T. vaginalis*, however, our results show that at lower (below 5) and higher (above 7) pH the adhesion indices are lower than at an intermediate pH.

The observation that parasites which were washed with PBS adhered to the substrate surface suggests that there are surface components which are part of the plasma membrane and which are involved in the process of adhesion. Previous studies suggested that a concanavalin A-binding glycoprotein located on the parasite's surface is involved in the process of attachment of *T. vaginalis* to the substrate (Cappuccinelli et al., 1975).

As previously described for vertebrate cells (Reviews in Curtis, 1966; Grinell, 1978), divalent cations such as Ca^{++} and Mg^{++} potentiate the adhesion of *T. vaginalis* to the substrate. However, this effect was clearly seen only with parasites of the Jt strain.

Our observations confirm previous studies which showed that microfilaments are involved in the process of adhesion of *T. vaginalis* to the substrate (Cappuccinelli et al., 1975). These observations suggest the presence of cytoplasmic microfilaments which might interact with the plasma membrane of the parasite.

Previous studies have shown that *Trichomonas vaginalis* has a net negative surface charge which results mainly from sialic acid residues associated to glycoproteins and glycolipids (Silva Filho et al., 1986). Our observations show that removal of sialic acid residues exposed on the parasite's surface significantly decrease the adhesion of the parasites to the substrate. This effect was observed with the three strains of *T. vaginalis* analysed. Previous studies have shown that incubation of *T. vaginalis* in the presence of neuraminidase reduces their surface charge in 35 to 50% (Silva Filho et al., 1986). This observation is in agreement with previous studies which show that neuraminidase treatment decreases the adhesion of mamalian tissue cells to glass (Weiss, 1973). At the other hand, studies carried out with leucocytes did not show a correlation between surface charge and cell adhesion. Reduction in the surface charge of the cells did not increase the adhesion to the substrate. However, cells treated with some chemotactic agents had their adhesion rate increased although they did not change their surface charge (Hoover et al., 1980). The results obtained up to now do not enable us to determine if the presence of surface sialic acid residues facilitates the adhesion of the parasites to the substrate as consequence of electrostatic interaction or as consequence of the fact the sialic acid residues may mask other carbohydrates residues, such as galactose and N-acetyl-galactosamine, which do not favour the interaction of the cell surface with the substrate. The results obtained when parasites are incubated in the presence of poly-L-lysine favour the idea that the surface charge plays an important role in the process of adhesion of *T. vaginalis* to the substrate. It is expected that poly-L-lysine blocks most of the anionic sites exposed on the parasite's surface, exposing its own cationic groups. Indeed parasites treated with poly-l-lysine presented a net positive surface charge, as determined by cell electrophoresis.

RESUMO

Papel de cátions divalentes, pH, componentes de citoesqueleto e carga de superfície na adesão de *Trichomonas vaginalis* a um substrato de poliestireno – O processo de adesão de três cepas de *Trichomonas vaginalis* a um substrato de poliestireno foi estudado. Verificou-se que este processo depende do tempo de incubação e do pH da solução salina em que os parasitos se encontram. A maior taxa de adesão foi observada após 60 minutos de incubação a pH 6,6. A adesão é maior se Ca^{++} ou Mg^{++} for adicionado ao meio. Tratamento das células em citocalasina B, tripsina ou neuraminidase reduz a adesão enquanto tratamento com poli-L-lisina facilita esta adesão. Incubação dos parasitos ou esferas de poliestireno na presença de poli-L-lisina pro-

voca alterações importantes na carga de superfície.

Palavras-chave: *Trichomonas vaginalis* – adesão celular – componentes do citoesqueleto – carga de superfície

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REFERENCES

BRASSEUR. P. & SAVEL, J., 1982. Evaluation de la virulence des souches de *Trichomonas vaginalis* par l'étude de l'effet cytopathogène sur culture de cellules. *C.R. Soc. Biol., 176* :849-860.

CAPPUCCINELLI, P.; CAGLIANI, I. & CAVALO, O., 1975. Involvement of a surface Concanavalin A-binding protein in the adhesion of *Trichomonas vaginalis* to substrates. Experimentia, 31 :1157-1159.

CAPPUCCINELLI, P. & VARESIO, L., 1975. The effect of cytochalasin B, colchicine and vinblastine on the adhesion of *Trichomonas vaginalis* to glass surfaces. *Inst. J. Parasitol., 5 :57-61.*

CURTIS, A.S.G., 1966. Cell adhesion. *Science Progress Oxford 54* :61-68.

DIAMOND, L.S., 1957. The establishment of various trichomonas of animals and man in axenic cultures. *J. Parasitol., 43* :488-490.

GRINNEL, F., 1978. Cellular adhesiveness and extracellular substrata. *Int. Rev. Cytol., 53* :65-144.

HONIGBERG, B.M., 1978. Trichomonads of importance in human medicine, p. 275-454. In J.P. Kreier, *Parasitic Protozoa.* Academic Press, New York.

HONIGBERG, B.M., 1979. Biological and physiological factors affecting pathogenicity of trichomonads, p. 409-427. In: M. Lavandowsky & S.H. Hutner, *Biochemistry and Physiology of Protozoa,* Academic Press, New York.

HOOVER, R.L.; FOLGER, R.; HARRING, W.A.; WARE, B.R. & KARNOVSKY, M.J., 1980. Adhesion of leukocytes to endothehum: role of divalent cations, surface charge, chemotactic agents and substrate. *J. Cell Sci., 45* :73-86.

LUMSDEN, W.H.R.; ROBERTSON, D.H.H. & MCNEILLAGE, G.J.C., 1966. Isolation, cultivation, low temperature preservation, and infectivity titration of *Trichomonas vaginalis. Brit. J. Ven. Dis., 42* :145-154.

PHILLIPS, H.J. & TERRYBERRY, J.E., 1957. Counting actively metabolizing tissue cultured cells. *Exp. Cell. Res., 13* :341-347.

SILVA FILHO, F.C.; ELIAS, C.A. & DE SOUZA, W., 1982. The surface charge of *Tritrichomonas foetus. J. Protozool., 29* :551-555.

SILVA FILHO, F.C.; ELIAS, C.A. & DE SOUZA, W., 1985. Further studies on the surface charge of various strains of *Trichomonas vaginalis* and *Tritrichomonas foetus. Cell Biophys., 8* :161-176.

WARTON, A. & HONIGBERG, B.M., 1983. Analysis of surface saccharides in *Trichomonas vaginalis* strains with various pathogenicity levels by fluorescein – conjugated plant lectins. *Z. Parasitenk., 69* :149-159.

WEISS, L., 1973. Neuraminidase, sialic acids, and cell interactions. *J. Nat. Cancer Inst., 50* :3-19.