Linear inactivation curves are obtained by plotting on a semilog scale the viable cell number against the exposure time. The LD_{50} ranged from 40 to 112 sec. The different shape of the curves could be connected with different factors, such as either a different transparency of the external cell layers to U.V. light, or differences at the nuclear level (e.g. the nuclear ploidy or the number of nuclei per colony forming unit).

The percentage of R-type colonies present on plates of Strain Z-1 after U.V. treatment does constantly increase in proportion to the duration of the treatment, thus indicating that metabolic changes, presumably due to U.V.-induced mutations in the host genome, may interfere with the expression of the host-parasite interrelationship. Further suggestion of a metabolic complementarity between MLO and its host, likely at the protein synthesis level, arose also during attempts to eliminate the presence of MLO from infected strains by treatment with antibiotics effective against mycoplasmas, e.g. erytromycin, kanamycin, lincomycin, spiromycin, and tetracyclines. Evidence is available that tetracycline does inhibit the microfungus strain according to whether MLO is invasive, latent, or absent (work in progess).

As far as the dimensions of Giemsa-positive bodies are concerned, the strains here studied have the diameters and volumes summarized in the Table.

Two different populations of Giemsa-positive bodies have been observed in Strain Z-1, having diameters of 0.9 μm (fungal nuclei, several nuclei per cell as in Strains 2-1 and R-1) and 0.3 μm on the average. More than 1 dozen of the small bodies (an electron microscopic picture of these bodies is reported in Figure 5) may be present in 1 fungal cell, their presence having a probable connection with the MLO infection. In cells of R-type strains their number is significantly reduced, more than 70% (unpublished data).

As a concluding remark, the present data seem to support the evidence that an interplay does take place between the nuclear characters of the host strain and the invasiveness and pathogenicity of the MLO.

Summary. The infection of MLO in a soil microfungus (*Humicola* sp.) originates different types of non-lethal parasitism. Differences have been found in the U.V. sensitivity and nuclear characters according to the type of MLO-microfungus interrelationship.

A. A. Lepidi, C. Filippi, Manuela Giovannetti and M. P. Nuti[6]

Istituto di Microbiologia agraria e tecnica dell'Università di Pisa, Via S. Michele 6, I-56100 Pisa (Italy), 1 April 1975.

[6] The authors express their thanks to Professor G. Picci for helpful discussion of the typescript. The skillful technical assistance of Mr. V. Gherarducci and Mr. G. Mela is gratefully acknowledged.

Involvement of a Surface Concanavalin A-Binding Glycoprotein in the Adhesion of *Trichomonas vaginalis* to Substrates

Trichomonas vaginalis cells will adhere to various types of solid support[1]. When this occurs on the walls of culture vessels, monolayer cultures not unlike those of multicellular organisms, are formed[2]. Adhesiveness is probably dependent on the properties of the cell membrane and many lines of evidence indicate that, in eucaryotic cells, surface glycoproteins are involved in adhesion phenomena[3].

A study was therefore made on the *T. vaginalis* surface to investigate the role of carbohydrate-containing proteins in cell-to-glass adhesion.

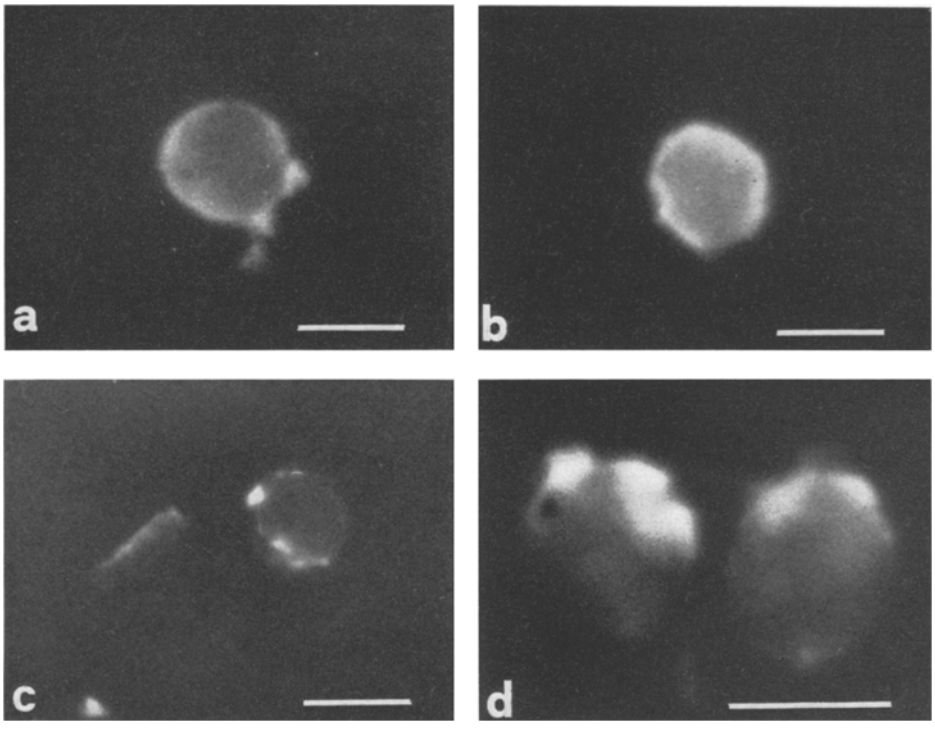
Materials and methods. The *Trichomonas* strain employed (FC) was maintained axially as previously described[2]. Only 12-h cultures containing $>98\%$ viable cells, as determined by trypan blue exclusion test[4], were used.

To determine binding of the jack bean lectin concanavalin A (ConA) to the surface of *T. vaginalis*, an indirect immunofluorescent test was performed[5]. Washed cells (4×10^4/ml in 0.14 *M*, pH 7.0 phosphate buffered saline – PBS) were incubated with excess ConA (Pharmacia Fine Chemicals AB; batch No. 7623) for 15 min at 37 °C and, after repeated washing, with a fluorescein isothiocyanate (FITC)-labelled immunoglobulin fraction of anti-ConA rabbit antiserum. Further washing was followed by resuspension in PBS and observation under a Leit Ortolux microscope with transmitted UV-light.

The ethylene diaminetetraacetic acid (EDTA) treatment of *Trichomonas* cells was performed as previously described[6]. The EDTA soluble fraction obtained (EDTA-SF) was then separated by affinity chromatography with ConA covalently bound to Sepharose-4B. 20 ml of EDTA-SF containing 35 mg of protein was dialyzed against 0.1 *M* sodium acetate buffer, pH 6.0 containing 1 *M* NaCl, 0.001 *M* $CaCl_2$, 0.001 *M* $MgCl_2$ and 0.001 *M* $MnCl_2$. The solution was then applied to a column of ConA-Sepharose (Pharmacia Fine Chemicals AB; batch No. 6745), bed volume 48 ml, and eluted with the above buffer. After the unbound fraction was collected (ConA-UF), elution with α-methyl-D-glucopyranoside gave a fraction (ConA-BF) that proved homogeneous on sodium dodecyl sulphate-(SDS)-polyacrylamide gel electrophoresis and was apparently a glycoprotein since it was stainable with Coomassie blue and PAS-positive[7].

Results and discussion. The distribution of ConA receptors on the surface of normal *T. vaginalis* is shown in the Figure a; fluorescence pattern is uniform with dots of varying intensity distributed over the entire cell surface. The appearance of fluorescence on the membrane is specific, since it is completely inhibited when ConA is pre-incubated with 0.1 *M* α-methyl-D-glucopyranoside as a hapten inhibitor.

[1] W. H. R. Lumsden, D. H. H. Robertson and G. J. C. McNeillage, Br. J. ven. er. Dis. *42*, 145 (1966).
[2] P. Cappuccinelli, C. Lattes and I. Cagliani, Atti 16th Congr. Soc. ital. Microbiol. (Giardini Editori, Pisa 1972), vol. 3, p. 411.
[3] R. B. Kemp, C. W. Lloyd and G. M. W. Cook, in *Progress in Surface and Membrane Science* (Eds. J. F. Danielli, M. D. Rosenberg and D. A. Cadenhead; Academic Press, New York 1973), vol. 7, p. 271.
[4] T. Takahashi, L. J. Old and E. A. Boyse, J. exp. Med. *131*, 1325 (1970).
[5] P. M. Comoglio and R. Guglielmone, FEBS Lett. *27*, 256 (1972).
[6] P. Cappuccinelli, S. Scannerini and A. Negro-Ponzi, G. Batt. Immun. *66*, 176 (1973).
[7] R. M. Zacharius, T. E. Zell, J. H. Morrison and J. J. Woodlock, Analyt. Biochem. *30*, 148 (1969).



Distribution of ConA receptors on the surface of *T. vaginalis*. Insert scale shows 10 μm.
a) *Trichomonas* treated with ConA for 15 min and incubated with a FITC-labelled immunoglobulin fraction of rabbit anti-ConA antiserum. The cells display uniform 'ring' fluorescence. b) as before but 50 μg/ml cytochalasin B was added. The labelled cells show uniform fluorescence. c) and d) as in a) but preceded by incubation with 0.005 *M* EDTA in PBS 0.14 *M* for 15 min and maintenance with 10 μg/ml cycloheximide for the duration of the experiment. Patchy fluorescence can be observed.

Drugs that damage microtubular structures within the cell[8] and inhibit lectin-induced agglutination of tumour cells[9], namely vinblastine (Lilly – 30 μg/ml) or cytochalasin B (Imperial Chemical Industries, UK – 50 μg/ml) had no effect on this pattern (Figure b).

When the experiment is repeated with EDTA-treated cells and a protein synthesis inhibitor (10 μg/ml cycloheximide –CH) is added, fluorescence is reduced and ConA appears distributed in irregular spots over the cell surface (Figure c–d). Treatment with EDTA, in fact, is known to cause membrane alterations of both normal and transformed cells[10,11] and to modify the distribution of ConA on the surface of cultured cells[12]. It has also been shown that a short treatment with EDTA inhibits *Trichomonas* adhesion for about 4 h, a period that can be prolonged indefinitely by contact with a protein synthesis inhibitor[13]. Furthermore, electron microscopic studies of EDTA-treated *Trichomonas*[6], have demonstrated a release of cell membrane components which may account for the modification of fluorescence pattern observed here.

To demonstrate that ConA-BF was specifically involved in *T. vaginalis* adhesion, EDTA-treated protozoa were maintained in Earle's solution at 37 °C in the presence of 10 μg/ml cycloheximide (Sigma). Under these conditions, there is little tendency for the cells to adhere to glass surfaces. However, when the ConA-BF was added adhesion was resumed, though less efficiently than in the controls (Table). Incubation of similarly treated cells with the ConA-UF or with bovine serum albumin (BSA) did not have this effect. The activity of the ConA-BF is specific. It is inhibited if the EDTA-treated cells are first incubated for 15 min in Earle's solution containing 2% inactivated anti-*Trichomonas* rabbit serum (anti-TS). Antibodies very probably prevent attachment of ConA-BF by clinging to the cell membrane.

Effect of a ConA-binding fraction on the adhesion of EDTA and cycloheximide treated *T. vaginalis* to glass surfaces (percentage of controls)

Trichomonas treatment	Added fraction (μg/ml protein, final concentration)			Adherent cells after 1 h (%) (mean ± SD)
	ConA-BF	ConA-UF	BSA	
EDTA + CH	2			51 ± 5
EDTA + CH	10			63 ± 6
EDTA + CH		2		11 ± 3
EDTA + CH		10		10 ± 2
EDTA + CH			30	14 ± 3
EDTA + CH	10	10	10	65 ± 9
EDTA + CH				7 ± 2
EDTA + CH + anti-TS	15			12 ± 3
Untreated controls				100 ± 8

Results compiled from 7 experiments. For abbreviations see text. *Trichomonas* were incubated with 0.005 *M* EDTA in 0.14 *M*, pH 7.0 PBS for 15 min at 37 °C, washed and diluted to a final concentration of 4×10^4 cells per ml in Earle's solution containing 10 μg/ml cycloheximide and the indicated amount of added fraction. Cell adhesiveness to glass was measured as previously described[17].

[8] K. G. BENSCH and S. E. MALAWISTA, J. Cell Biol. *40*, 95 (1969).
[9] N. K. WESSELS, B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUENA, E. L. TAYLOR, J. T. WRENN, K. M. YAMADA, Science *171*, 135 (1971).
[10] C. SNOW and A. ALLEN, Biochem. J. *119*, 707 (1970).
[11] L. A. CULP and P. H. BLACK, Biochemistry *11*, 2161 (1972).
[12] C. HUET and M. HERZBERG, J. Ultrastruct. Res. *42*, 186 (1973).
[13] P. CAPPUCCINELLI, I. CAGLIANI and G. CAVALLO, *Progress in Protozoology* (Eds. P. DEPUYTORAC and J. GRAIN; U.E.R., Clermont-Ferrand 1973), p. 77.

It has been shown that cell surface carbohydrates are involved in both intercellular and substrate adhesion on the part of procaryotic and eucaryotic cells[14,15], though no clear relation between adhesion and ConA-binding sites has yet been demonstrated[3,16].

Our findings, however, suggest that the carbohydrate-containing surface protein that binds ConA is primarily responsible for the adhesion of *T. vaginalis* to glass surfaces. The influence of other factors, however, such as the active protrusion of filopodia, or movements of the cell periphery mediated by cytochalasin B-sensitive microfilaments, must not be overlooked[17].

These results are in line with the hypothesis that sugar-containing proteins are involved in cellular interactions and particularly in cell-to-substrate adhesion phenomena[3,15,18,19].

Summary. Treatment of *Trichomonas vaginalis* with EDTA removes their ability to adhere to glass surfaces and changes their affinity to Concanavalin A (ConA) by a different distribution of their surface structures. Filtrates of the EDTA-treated *Trichomonas* passed through affinity chromatography columns (ConA bound to Sepharose 4B) separate into 2 fractions, one fraction was bound to the ConA-Sepharose beads, the other was not. The Con A - bound fraction appears to be a glycoprotein which restores in a specific way the ability of the EDTA-treated protozoa to adhere to glass.

P. Cappuccinelli, Irene Cagliani and G. Cavallo[20]

University of Torino, School of Medicine, Institute of Microbiology, Via Santena 9, I 10126 Torino (Italy),
4 November 1974.

[14] M. Fletcher and G. D. Floodgate, J. gen. Microbiol. *74*, 325 (1973).
[15] E. J. McGuire, in *Membrane Research* (Ed. C. F. Fox; Academic Press, New York 1972) p. 347.
[16] M. S. Steinberg and I. A. Gepner, Nature New Biol. *241*, 249 (1973).
[17] P. Cappuccinelli and L. Varesio, Int. J. Parasit. *5*, 57 (1975).
[18] B. Gessner and L. Thomas, Science *151*, 590 (1966).
[19] M. M. Burger, Proc. natn. Acad. Sci., USA *62*, 994 (1965).
[20] We want to thank Dr. P. M. Comoglio for the donation of the FITC-labelled immunoglobulin fraction of anti-ConA rabbit antiserum. This work was supported by a grant from the Italian National Research Council (C.N.R.).

Environmental Factors Control the Periodical Reproduction of Tropical Electric Fish

Weakly electric fish (Gymnotoidei, Mormyriformes) have since the time of their discovery[1] been more and more the object of intensive studies[2]. Two important topics, however, sexual behaviour and embryology, could not as yet be studied systematically.

Little is known about the reproduction of these fishes. Apart from an isolated chance success with *Petrocephalus bovei*[3], weakly electric fish have never been bred in captivity. The Gymnotoidei, which live in tropical Central and South America, breed during the rainy season[4] as do most of the African mormyrid fishes[5,6]. The factors which control the periodical reproduction of fishes in the tropics in relation to dry and rainy season were, however, not known[7]. It has not therefore been possible up to now to induce gonad growth in these fishes in the laboratory.

Eigenmannia virescens was chosen for most of the experiments because they are transparent and the growth of the gonads could be continuously observed. A prolonged series of experiments has proved that a combination of environmental factors induce the growth of gonads in *Eigenmannia:* pH and conductivity of the water were continuously decreased. At the same time, the water level was increased. Simulated rain (8 h/day) and a constant photoperiod (L D 13:11) completed the system. After about 2 months under these conditions, the animals showed mature gonads (Figure 1). These fish are similar in appearance to mature fish caught in South America during the rainy season[4]. If the above conditions are changed in the opposite direction, then mature *E. virescens* reduce their gonads in 4 to 6 weeks.

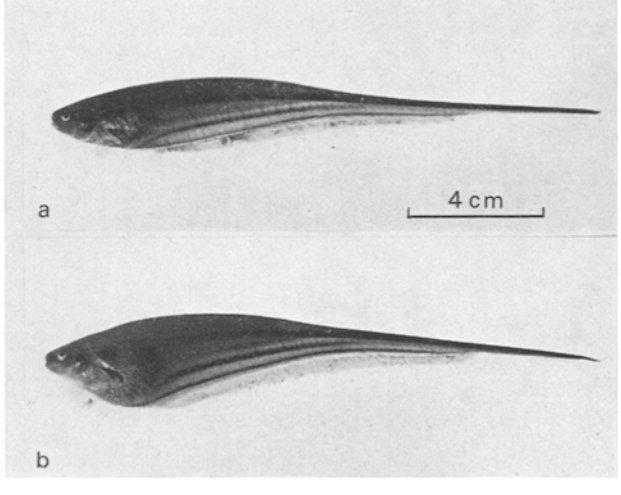


Fig. 1. Female of *E. virescens* with entirely reduced ovary (a), and the same fish 87 days later after induction of ovarian growth (b) by simulating the rainy season (Figure 2).

[1] H. W. Lissmann, Nature, Lond. *167*, 201 (1951). – C. W. Coates, M. Altamirano and H. Grundfest, Science *120*, 845 (1954).
[2] M. V. L. Bennett, in *Fish Physiology* (Eds. W. S. Hoar and D. J. Randall; Academic Press, New York and London 1971), vol. 5, p. 347; and vol. 5, p. 493. – T. Szabo, in *Handbook of Sensory Physiology* (Ed. A. Fessard; Springer Verlag, Berlin, Heidelberg, New York 1974), vol 3, p. 13; A. Fessard, T. Szabo, ibid. p. 59; Ad. J. Kalmijn, ibid. p. 147; H. Scheich, T. H. Bullock, ibid. p. 201.
[3] J. Birkholz, *Das Aquarium 3*, 201 (1969); and *4*, 340 (1970).
[4] M. M. Ellis, Mem. Carnegie Museum *6*, 109 (1913). – K. D. Hopkins, Behaviour *50*, 270 (1974).
[5] J. Blache, *Les poissons du bassin du Tchad et du bassin adjacent du Mayo Kebbi* (O.R.S.T.O.M., Paris 1964), pp. 27–58. – G. Nawar, Ann. Mag. nat. Hist. ser. 13, *2*, 603 (1960); and 493 (1960).
[6] J. Okedi, Revue Zool. Bot. Afric. *79*, 34 (1969).
[7] H. O. Schwassmann, in *Fish Physiology* (Eds. W. S. Hoar and D. J. Randall; Academic Press, New York and London 1971), vol. 6, p. 371.