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Ethanol Affects Endocytosis and Proliferation of *Tetrahymena pyriformis* GL and Promotes Encystment

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Summary. Exponentially multiplying cells were exposed to 0.25-2.5 % ethanol (v/v) in the growth (PP) medium. Acute exposure was studied during a 6-h period, i.e. two normal cell generations, whereas chronic exposure was determined after 24 h, or more, in ethanol. The effects of low ethanol concentrations (0.25-1.0 %) were modest as the rate of endocytosis was almost normal and the rate of cell proliferation only slightly decreased. In high concentrations (>1.0 %) the acute effects of ethanol were dose- and time-dependent with respect to a lag period before proliferation resumed at a decreased rate and to the capacity of cells to form food vacuoles. After 24 h in the low ethanol concentrations the cells appeared to have adapted. In the high concentrations, however, chronic effects of ethanol were a much decreased rate of endocytosis and a distinct separation of the cells into two populations: normal sized cells and small mostly round cells. The small round cells were lying at the bottom af the culture dish, motionless or slowly moving with no visible oral structure but with somatic cilia, some cells lacked cilia and had a refractive outline or layered wall structure. These cells looked like precystic stages among which a few resting cysts were seen with pores in the cyst wall. Promotion of encystment in *Tetrahymena pyriformis* is surprising as the phenomenon is not considered a feature of this organism.

Key words: encystment, ethanol, growth, phagocytosis, *Tetrahymena pyriformis* GL-C.

INTRODUCTION

Exposure to ethanol is probably greater than to any other solvent, apart from water (Andrews and Snyder 1986). As a solvent ethanol is used widely for water-insoluble substances in industry and medicine but it is also a component of various intoxicating beverages. The effect of ethanol is dose- and time-dependent and chronic exposure may lead to serious conditions, such as a fatty liver (Andrews and Snyder 1986).

Ethanol is often considered as a “stimulant” but it is in fact an inhibitor (Albert 1981). The general effect of alcohol consumption is well known, it ranges from mild animation over impaired muscular coordination to loss of consciousness related to the level in blood. The mechanism by which alcohol causes these effects is not fully understood as many factors are involved (Andrew and Snyder 1986, Kranzler 1995). Pharmacological and toxi-

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cological effects of ethanol relate to the fact that it acts as both a general anesthetic and a nutrient as it may be metabolized by most organisms. As an anesthetic, ethanol causes a dose-dependent central nervous system depression (Andrews and Snyder 1986) which may be ascribed to lipid changes in the neuronal membrane (Moring and Shoemaker 1995) with displacement of proteins, i.e. an altered membrane functioning. Acute toxicity of ethanol may, however, be decreased by the presence in cells of heat shock proteins (HSP), i.e. stress proteins, which prevent protein degradation (Kampinga 1993) and ethanol is an inducer of HSP synthesis (Lepock *et al.* 1988).

The ciliate *Tetrahymena* is used as a model cell system in cytotoxicology and is highly adaptable to environmental stress (e.g. Nilsson 1989). A common reaction of cells to various stress conditions is induced changes in the membrane lipid composition as seen on exposure to ethanol (e.g. Moring and Shoemaker 1995). In *Tetrahymena*, chronic exposure, i.e. several days, to a high concentration of ethanol (~ 0.35 M) induced distinct changes in the membrane phospholipid composition (Nandini-Kishore *et al.* 1979, Goto *et al.* 1983).

In the present study *T. pyriformis* GL, an amicronucleate species, was exposed to different concentrations of ethanol (44-445 mM). The acute effect of ethanol on endocytosis and cell proliferation was studied during a 6-h period, i.e. 2 normal cell generations, and chronic effects were observed after 24 h and later. That ethanol affected cell proliferation is in agreement with reported findings by Nindini-Kishore *et al.* (1979). However, a most unexpected finding was that high ethanol concentrations promoted encystment, a phenomenon not generally recognized a feature of *T. pyriformis* (Furgason 1940, Corliss 1973).

MATERIALS AND METHODS

Tetrahymena pyriformis GL-C was grown axenically at 28°C in 2% proteose peptone (PP) enriched with 0.1% yeast extract and inorganic salts, pH 7.0 (Plesner *et al.* 1964). Batch cultures were maintained in 7 ml PP medium in test tubes. For experiments 100-ml cultures in 500-ml Fernbach flasks were agitated and aerated. Two cultures in the exponential growth phase (20-40,000 cells/ml) were mixed and divided into 50-ml cultures of which 3 received different concentrations of ethanol (v/v) and the 4th control culture received distilled water in a volume corresponding to that in which ethanol was added to the other cultures. Addition of ethanol did not change the pH of the medium. To reduce loss of the volatile ethanol the experimental cultures were not aerated or agitated although agitation of the cultures had little influence on the data as compared to those obtained without agitation; however, aeration made a great difference. The cultures were followed for 24 h or more.

The cells were also exposed to ethanol under starvation conditions in an inorganic salt (IM) medium (Plesner *et al.* 1964) to which the cells were transferred 1 h before addition of ethanol (3 experiments). Moreover, the cells were exposed to beer (Carlsberg lager, 4.6 % ethanol = 1 M; pH 4.3) after dilution with IM medium to 1.75 % ethanol and adjustment of pH to 7.2 before addition of concentrated cells. Concentrated cells were obtained by spinning down exponentially multiplying cells in a hand centrifuge, removal of the supernatant (PP medium), and refilling to the original volume with diluted beer; the control culture was treated similarly but refilled with PP medium diluted with IM in the same proportion as beer (4 experiments).

The cell density was determined electronically (Coulter Multisizer II, Coulter Electronics Ltd., England). Triplicate 0.5-ml cell samples were withdrawn from the cultures at hourly intervals and fixed in an equal volume of 1% glutaraldehyde in phosphate buffer (pH 7.2). The samples were diluted with 0.45% NaCl prior to counting.

The endocytic capacity was determined by a 10 min exposure of a 2-ml cell sample to 2 ml carmine particles (0.4 mg/ml) suspended in the same medium as that of the cells. At the end of the exposure the cells were fixed in 4 ml 1% buffered glutaraldehyde. After wash in distilled water, the number of labelled vacuoles was counted in 100 cells (Nilsson 1976). The endocytic capacity of the treated cell populations was expressed as the percent of that of control cultures (100%).

RESULTS

Proliferation and endocytosis

Exponentially multiplying cells were exposed to 0.25 - 2.5 % (v/v) ethanol (44 - 445 mM) in the growth, proteose peptone (PP), medium without aeration and agitation to minimize evaporation of the ethanol. The acute effect on cell proliferation is shown in Fig. 1 for a 6-h period, i.e. two normal cell generation times. A dose-dependent reduction of the increase in cell density (compared with the control) was seen after 1 h, i.e. the division of cells most advanced in the cell cycle. Cells in 0.25 and 0.5 % ethanol continued proliferation at slightly decreased rate without a lag period, whereas cells in 1, 1.5, and 1.75 % ethanol exhibited lag periods of 2, 3, and 5 h, respectively, before resuming proliferation at a decreased rate. Cells in 2 and 2.5 % (not shown) ethanol did not proliferate during the 6 h period. After 24 h and dose-dependently, cells in 0.25, 0.5, and 1 % had reached maximum cell density (~ 1,000,000 cells/ml), i.e. doubled 5-6 times, indicating adaptation to ethanol. The cell density in 1.5 and 1.75 % ethanol increased only 2.5 and 1.4 times after 24 h, respectively, i.e. cells in 1.75 % did not complete a full cell doubling. After 24 h in 2 % and

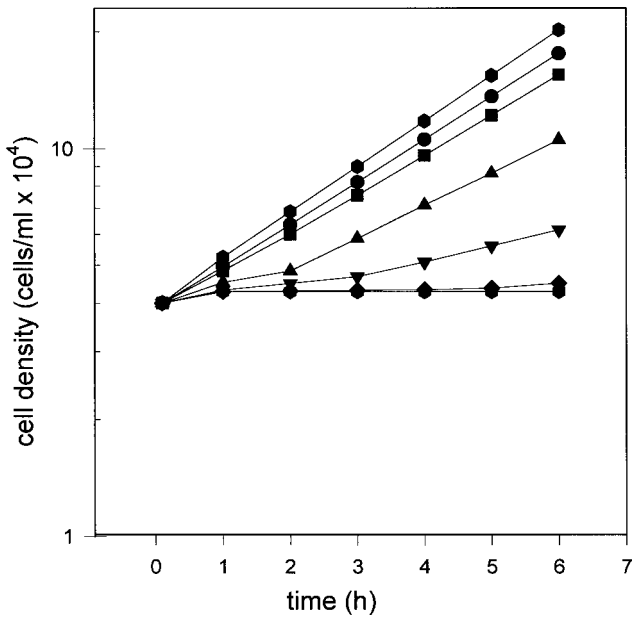


Fig. 1. The dose- and time-dependent effect of ethanol on proliferation of *Tetrahymena* during a 6-h exposure in proteose-peptone medium. Control (top solid hexagon), 0.25 % (solid circle), 0.5 % (solid square), 1 % (solid triangle), 1.5 % (solid upside-down triangle), 1.75 % (solid diamond), and 2 % (lower solid hexagon) ethanol. Means of 4-12 experiments per ethanol concentration. S.D. < 10 %.

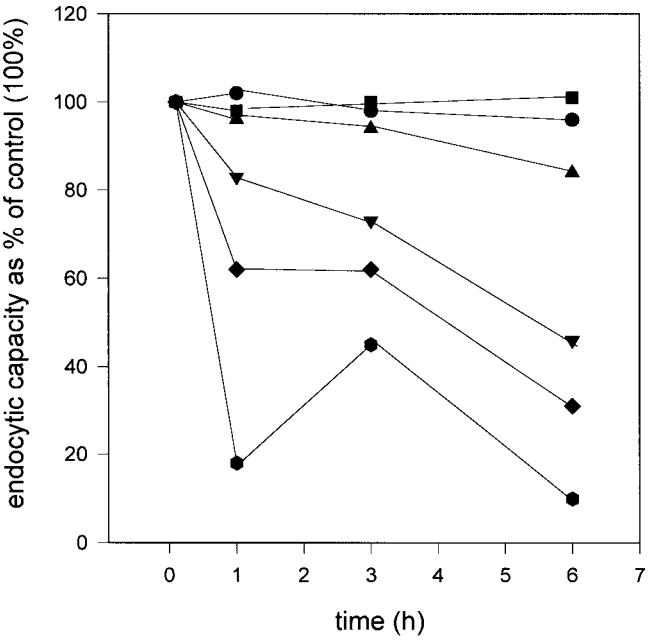


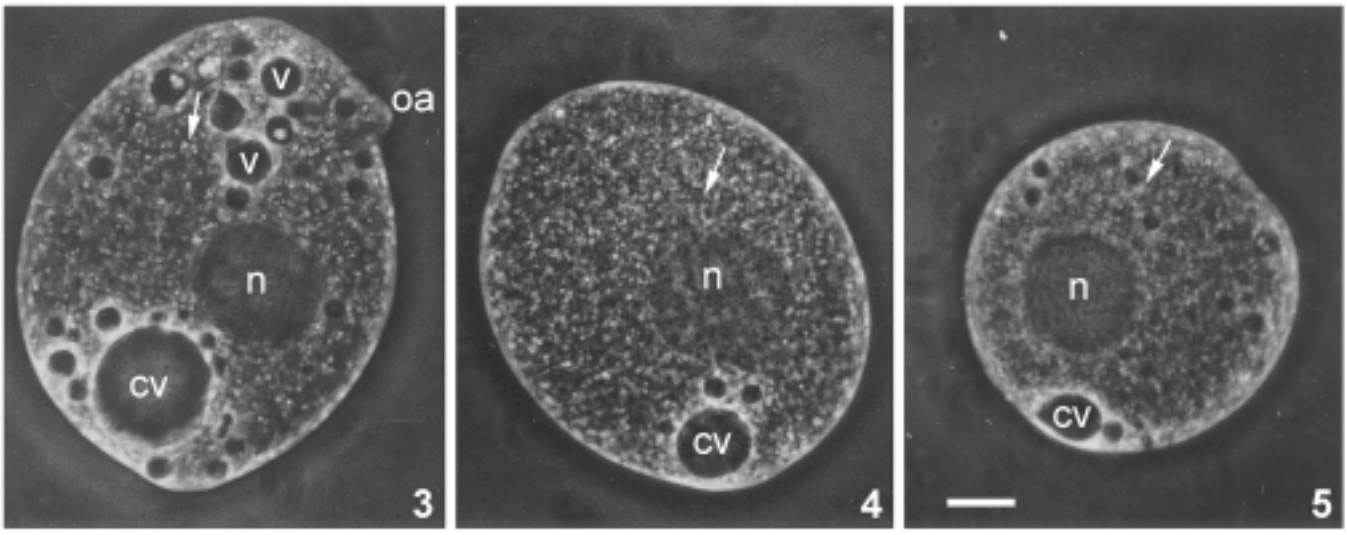
Fig. 2. The dose- and time-dependent effect of ethanol on the capacity of *Tetrahymena* to form food vacuoles (endocytosis) during a 6-h exposure in proteose-peptone medium. The data are expressed as the percent of the capacity of control cells (100 %). Cells in 0.25 % (solid circle), 0.5 % (solid square), 1.0 % (solid triangle), 1.5 % (solid upside-down triangle), 1.75 % (solid diamond), and 2.0 % ethanol (solid hexagon). Means of 4-12 experiments per ethanol concentration. S.D. < 10 %.

2.5 % ethanol the cell density decreased more than 20 % due to cell deaths. Hence, chronic exposure to ethanol revealed cell adaptation in the low concentrations but severely affected cells with deaths in the high concentrations. The highest sublethal concentration (without cell deaths) is therefore 1.75 % (~ 300 mM) ethanol in the PP medium.

The capacity of the cells to form food vacuoles (endocytosis) in the presence of ethanol, is shown in Fig. 2 for a 6-h period as a percentage of that of control cells (100 %). The rate of endocytosis was unaffected in 0.25 and 0.5 % ethanol, slightly decreased in 1 %, and time- and dose-dependently affected in 1.5, 1.75, and 2 % ethanol; the low 1-h values indicate a strong initial reaction to ethanol. Cells in 2.5 % ethanol did not form any food vacuoles, i.e. the rate follows the abscissa. After 24 h the rate of endocytosis increased 10-20 % above the control value (100 %) in 0.25 - 1 % ethanol, whereas it decreased to 11, 3, and 0 % in 1.5, 1.75, and 2 % ethanol, respectively.

The cells were also exposed to 0.5, 1.0, and 1.5 % ethanol in the absence of nutrients in an inorganic salt (IM) medium. The only increase in cell density was division of the cells most advanced in the cell cycle at addition of ethanol; this portion of the cells was affected dose-dependently as also seen after 1 h in PP medium (see Fig. 1). Endocytosis was affected, i.e. the starved cells in 0.5, 1.0, and 1.5 % formed food vacuoles after 1 h at a rate of 96, 77, and 52 %, after 3 h at a rate of 92, 63, and 46 %, and after 5 h at a rate of 97, 62, and 25 %, respectively, of the control value (100 %); moreover, after 24 h the rate was 89, 61, and 3 %, respectively. These values are lower than the rates of endocytosis, shown in Fig. 2, for cells exposed to 0.5, 1.0, and 1.5 % ethanol in the growth medium. After 24 h many dead cells were seen in 1.0 and 1.5 % ethanol in the IM medium which means that *Tetrahymena* is less tolerant to ethanol in the absence of nutrients. The highest sublethal concentration in the starvation medium is 0.5 % ethanol.

To investigate the high sensitivity of starved cells further, *Tetrahymena* was exposed to another nutrient, i.e. lager beer, after dilution to 1.75 % ethanol and adjustment of pH. The cells behaved much like cells in



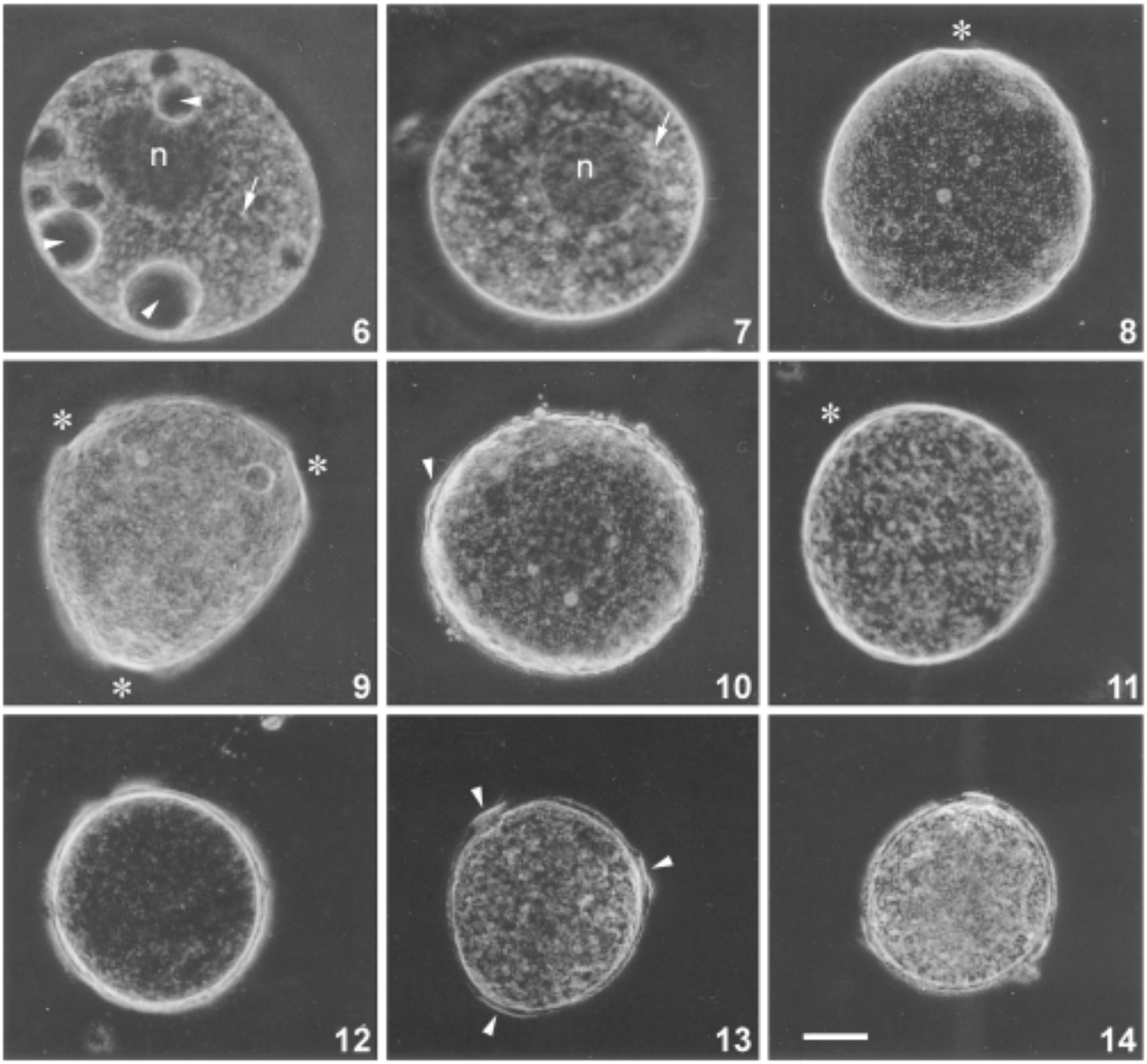
Figs 3-5. *In vivo* micrographs of compressed *Tetrahymena* exposed to 2 % ethanol in the growth medium. **3** - cell after 3.5 h exposure to ethanol; **4** - a cell from the "large cell" population after a 24 h exposure; **5** - a cell from the "small cell" population after a 24 h exposure. Nucleus (n); contractile vacuole (cv); food vacuoles (v); oral structure (oa); granules (arrows). Scale bar 10 µm.

the same concentration of ethanol in the PP medium (Fig. 1), i.e. a 5 h lag period, completion of less than one cell doubling after 24 h, and no cell death. The rates of vacuole formation after 1, 3, 6, and 24 h were 64, 65, 40, and 7 %, respectively, i.e. similar to the rates of cells in 1.75 % ethanol in the PP medium (Fig. 2). Hence, ethanol affects *Tetrahymena* identically in the two nutrient media, PP medium and beer, but starved cells only tolerate ethanol in lower concentrations.

Light microscopy

Light microscopical observation of ethanol-treated cells in the PP medium revealed normal cell behavior in the low concentrations. In the high ethanol concentrations, some cells lost their shape initially and became spherical with an enlarged contractile vacuole, concomitantly with the low 1 h values of phagocytosis (Fig. 2), but normal cell shape was restored by 3 h; this swelling of cells was also observed in the starvation medium. Small refractive granules appeared in all ethanol-treated cells, as typically seen in *Tetrahymena* during stress conditions (Nilsson 1976, 1989, 2003); the granules increased in number with time (Figs 3-5) and ethanol concentration. After 24 h, a distinct dose-dependent change occurred in the high ethanol concentrations (>1.5 %) as two distinct populations were seen, one of normal sized cells (uncompressed length/width = 1.7) and another of small, mostly spherical (uncompressed length/width = 1.1), cells (Figs 4, 5). This feature of two cell populations occurred also after 24 h in the ethanol-starvation medium and in beer. In PP medium, the number of small cells increased dose-dependently from a few % in 1.5 % ethanol and up to 90 % in 2.5 % ethanol after 24-48 h; hence, after 24 h in 1.75 % ethanol about 75 % of the cells were of normal size and shape, whereas the remaining 25 % were small, round cells lying at the bottom of the culture dish looking motionless, appearing dead.

On close observation, however, many of the small cells did move, not swimming in straight lines as the normal shaped cells but spinning around themselves. The cells were most often without visible oral structure or food vacuoles, only the macronucleus and contractile vacuole, or contractile vacuoles, were visible (Figs 5, 6). Transformation of pyriform cells to the small round precystic cells, was indicated by cells "dividing off" the pointed anterior end (round macronucleus remaining in the posterior end); the tiny triangular piece swam off leaving a round cell. Some cells did not move at all, they lacked cilia and did not have a contractile vacuole but they contained the macronucleus and numerous large granules (Fig. 7). Other cells were opaque, obscuring internal structures, and their smooth refractive outline showed indication of pore sites (Figs 8, 9). Precystic



Figs 6-14. *In vivo* micrographs of ethanol-induced encystment of *Tetrahymena*. Stages from the "small cell" population, after 24-48 h in 2 % ethanol in the growth medium, arranged in a possible sequential order. **6** - an early round precystic cell showing the macronucleus (n), several contractile vacuoles (arrow heads), and numerous granules (arrow); note, the absence of an oral structure; **7** - another spherical precystic stage with no sign of cilia; note, the macronucleus (n), the many large granules (arrow), and no contractile vacuole; **8** - a compact "cell" with a smooth, refractive outline and early indication of a pore site (star); **9** - probably a slightly later stage than that shown in the Fig. 8 with indication of pore sites (stars); **10** - a compact precystic stage with a rough, refractive outline due to extruded material for the cyst wall, note the indication of a double layered wall (arrow head); **11** - another compact stage within a thickened, refractive wall with indication of a pore site (star); **12** - an early cyst stage with a smooth, refractive double "shell"; **13** - a resting cyst showing 3 pore structures (arrow heads) in optical section and a layered cyst wall; **14** - another resting cyst with pore structures after 24 h in 1 % ethanol in the starvation medium. Scale bar 10 μm.

stages with an irregular outline due to extruded material (Fig. 10), probably for cyst wall formation as the refractive rim increased (Fig. 11) and thickened to form a less flexible (not compressible) early cyst with a layered smooth, refractive wall (Fig. 12); resting cysts had pores in the wall (Figs 13, 14). Although the sequential order of events is uncertain, the present findings seem to indicate that pore sites are created, or determined, prior to formation of the elaborate cyst wall; moreover, that the unusual appearance of several contractile vacuoles along the cell periphery (Fig. 6) may play a role in determining the site, or in creation, of the pores. The frequency of encystments was dose-dependent and highest in 2 % and 2.5 % ethanol but the yield of cysts was low, whereas the precystic stages were plentiful. No attempt was made to study or induce excystment.

As ethanol can induce encystment it means that the phenomenon must be a normal capacity of *T. pyriformis* and cysts should therefore occur also in old batch cultures in which the cells die out due to the increasing pH (> 8.5). Examination of the debris at the bottom of old batch (several days/weeks) cultures with a few swimming cells, revealed indeed numerous small round bodies, mostly ghosts but also moving cells, resembling the precystic stages induced by ethanol; resting cysts with pores were found but rarely.

DISCUSSION

Ethanol did not affect *Tetrahymena* much in low concentrations, the cells adjusted to up to 1 % (~175 mM) in the growth PP medium. In high concentrations, however, both phagocytosis and cell proliferation were affected dose- and time-dependently by ethanol. The present finding that ethanol affected cell proliferation, confirms the report by Nandini-Kishore *et al.* (1979) although the authors found growth of *Tetrahymena* in somewhat higher concentrations than seen in the present study. Chronic exposure of *Tetrahymena* to 1.6 % (w/v), or 0.35 M, ethanol caused profound changes in membrane fluidity (Nandini-Kishore *et al.* 1979, Goto *et al.* 1983), i.e. an altered membrane functioning, a feature which would affect the rate of endocytosis. Moreover, that ethanol affected vacuole formation in *T. pyriformis* with no, or little, inhibition in low concentrations and depression in high concentrations, resembles the increased activation of Kupffer cell phagocytosis in low, and depression in high, concentrations of ethanol (Eguchi *et al.* 1991). Another factor which may relate to the affected endocytosis in *Tetrahymena*, is that ethanol increases the $[Ca]_i$ in Kupffer cells which may disturb the phagocytic process (Hijioka *et al.* 1993). Incidentally, an increased $[Ca]_i$ in *Tetrahymena* would increase transport of excess calcium from the cytoplasm into the small refractive granules, i.e. also increase their number, to maintain homeostasis (see Nilsson 2003). That the endocytic capacity of starved cells was affected in a lower concentration of ethanol than in the nutrient media, could be ascribed to the fact that the starved cells were subjected to double stress: i.e. sudden transfer to starvation conditions followed by an exposure to ethanol.

The most surprising and unexpected effect of ethanol was the promotion of encystment in *T. pyriformis*, especially since cysts are not a recognized feature of this organism: “neither reproductive nor resting cysts are recorded” (Corliss1973). In the past, however, two reports on cysts have appeared. Watson (1946) described thin-walled, oval cysts formed after drying a *Tetrahymena* culture slowly on an agar plate; Corliss (1953) was unsuccessful in reproducing such cysts. Hurst (1957) reported in an abstract on the occurrence of “cysts” in *T. pyriformis* (variety 2): “the round, dark, sessile structures contained a micro- and a macronucleus but no nuclear reorganization was observed”, i.e. not enough information to identify the structure of the cysts (pores?).

Encystment is a common phenomenon in nature as a manner of survival. Free-living ciliates have seasonal blooms with interim periods of environmental stress (drought, frost, and snow) affecting their food supply so the ciliates must encyst to survive and often conjugation precedes encystment (e.g. Corliss and Esser 1974, Fenchel 1987). An arctic micronucleate *Tetrahymena* sp. isolated from a fresh-water pond in Greenland (Larsen 1992), is a typical ciliate living under extreme conditions. The ciliate was established in the laboratory in mixed cultures and cysts were commonly found in old cultures (unpublished observations), whether conjugation preceded encystment was not recorded. The cysts have pores in the wall and resemble the cysts (Figs 13, 14) induced by ethanol in *T. pyriformis*. The arctic *Tetrahymena* sp. probably belongs to the *T. pyriformis* complex (Corliss 1973, Nanney and McCoy 1976) which is not known to form cysts. Members of the *T. rostrata* complex are mostly parasitic species and form cysts readily. Descriptions of the cysts of *T. rostrata* (Corliss 1973, McArdle *et al.* 1980) do not mention pores in the cyst wall, hence they differ in structure from the *T. pyriformis* GL cysts described here.

Even though conjugation usually precedes encystment (e.g. Corliss and Esser 1974) it cannot be a prerequisite for formation of cysts since *T. pyriformis* GL can form cysts but is unable to conjugate. It was proven amicronucleate 65 years ago (Furgason 1940) and it has been maintained axenically in organic growth medium for even longer. Hence, it may be concluded from the present study that the genetic information of the micronucleus is not essential for encystment, or perhaps not entirely, as admittedly the yield of mature cysts was low whereas the yield of precystic stages was very high.

A last question is: why does ethanol promote encystment of *Tetrahymena pyriformis*? Without knowing the details, apart from the ethanol-induced changes in the membrane phospholipids (Nandini-Kishore *et al.* 1979, Goto *et al.* 1983), an answer may be that high concentrations of ethanol depress food vacuole formation in rapidly multiplying cells, i.e. the cells are abruptly brought to a state of starvation, a common trigger of encystment (Corliss and Esser 1974, Fenchel 1987).

REFERENCES

Albert A. (1981) Selective Toxicity. Chapman and Hall. London, New York

Andrews L. S., Snyder R. (1986) Toxic effects of solvents and vapors. In: Casarett & Doull's Toxicology (Eds. D. C. D. Klaassen, M.O. Amdur, J. Doull), 3rd ed. Macmillan Publ. Comp., New York, Toronto, London, 600-700

Corliss J. O. (1953) Comparative studies on holotrichous ciliates in the *Colpidium-Glaucoma-Leucophys-Tetrahymena* group. II. Morphology, life cycles, and systematic status of strains in pure culture. *Parasitology* **43:** 49-87

Corliss J. O. (1973) History, taxonomy, ecology, and evolution of species of *Tetrahymena*. In: Biology of *Tetrahymena* (Ed. A. M. Elliott). Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania, 1-55

Corliss J. O., Esser S. C. (1974) Comments on the role of the cyst in the life cycle and survival of free-living protozoa. *Trans. Amer. Micros. Soc.* **93:** 578-593

Eguchi H., McCuskey P. A., McCuskey R. S. (1991) Kupffer cell activity and hepatic microvascular events after acute ethanol ingestion in mice. *Hepatology* **13:** 751-757

Fenchel T. (1987) Ecology of Protozoa. The Biology of Free-living Phagotrophic Protists. Springer-Verlag, Berlin, New York, London, Paris, Tokyo

Furgason W. H. (1940) The significant cytostomal pattern of the "*Glaucoma-Colpidium* group", and a proposed new genus and species, *Tetrahymena geleii*. *Arch. Protistenk.* **94:** 224-266

Goto M., Banno Y., Umeki S., Kameyama Y., Nozawa Y. (1983) Effects of chronic ethanol exposure on composition and metabolism of *Tetrahymena* membrane lipids. *Biochim. Biophys. Acta* **751:** 286-297

Hijioka T., Goto M., Lemasters J. J., Thurman R. G. (1993) Effect of short-term ethanol treatment on voltage-dependent calcium channels in Kupffer cells. *Hepatology* **18:** 400-405

Hurst D. D. (1957) Occurrence of "cysts" in *Tetrahymena pyriformis*, variety 2. *J. Protozool.* **4:** Suppl. 18, abstract 73

Kampinga H. H. (1993) Thermotolerance in mammalian cells. Protein denaturation and aggregation, and stress proteins. *J. Cell Sci.* **104:** 11-17

Kranzler H. R. (1995) The pharmacology of alcohol abuse: an introduction. *Handb. Exp. Pharm.* **114:** 2-10

Larsen H. F. (1992) Süsswasserciliaten aus Grönland. Blepharisma, Tetrahymena und andere Arten. *Mikrokosmos* **81:** 297-302

Lepock J. R., Frey H. E., Rodahl A. M., Kruuv J. (1988) Thermal analysis of CHL V79 cells using differential scanning calorimetry: implications for hyperthermic cell killing and heat shock response. *J. Cell Physiol.* **137:** 14-24

McArdle E. W., Bergquist B. L., Ehret C. F. (1980) Structural changes in *Tetrahymena rostrata* during induced encystment. *J. Protozool.* **27:** 388-397

Moring J, Shoemaker W. J (1995) Alcohol-induced changes in neuronal membranes. *Handb. Exp. Pharm.* **114:** 12-53

Nandini-Kishore S.G., Mattox S. M., Martin C. E., Thompson, Jr. G. A. (1979) Membrane changes during growth of *Tetrahymena* in the presence of ethanol. *Biochim. Biophys. Acta* **551:** 315-327

Nanney D. L., McCoy J. W. (1976) Characterization of the species of the *Tetrahymena pyriformis* complex. *Trans. Amer. Micros. Soc.* **95:** 664-682

Nilsson J. R. (1976) Physiological and structural studies on *Tetrahymena pyriformis* GL. With special reference to endocytosis and to structural changes during the cell cycle and in response to alterations in the cellular environment. *C. R. Trav. Lab. Carlsberg* **40:** 215-355

Nilsson J. R. (1989) *Tetrahymena* in cytotoxicology: with speciel reference to effects of heavy metals and selected drugs. *Europ. J. Protistol.* **25:** 2-25

Nilsson J. R. (2003) How cytotoxic is zinc? A study on effects of zinc on cell proliferation, endocytosis, and fine structure of the ciliate *Tetrahymena*. *Acta Protozool.* **42:** 19-29

Plesner P., Rasmussen L., Zeuthen E. (1964) Techniques used in the study of synchronous *Tetrahymena*. In: Synchrony in Cell Division and Growth. (Ed. E. Zeuthen). Interscience Publ., New York, 543-563

Watson J. M. (1946) On the coprophilic habits of a ciliate - *Glaucoma piriformis*. *J. Trop. Med. Hyg.* **49:** 44-46

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