ments, and of cytoplasmic microtubules and/or membrane-bound tubulin in particular, for the morphogenesis[10], functioning and control of smooth endoplasmic reticulum in arterial SMC, and in hepatocytes? In this respect, a number of microtubule-disassembling agents (colchicine, nocodazol, etc.), microtubule-stabilizing agents (taxol) and other agents which may influence tubulin-microtubule equilibrium (calmodulin antagonists such as trifluoperazine, etc.), may be used as experimental tools for the study of smooth endoplasmic reticulum in different kinds of cells. Some recent data have demonstrated that colchicine may increase the acetylcholine-induced contraction of stomach strips in vitro[4] and the contraction of cardiomyocytes in culture[11], and it may decrease the duration of hexobarbital-induced sleep in rats[5] and may alter the activity of some microsomal drug-metabolizing enzymes in liver[6]. Certainly, studies with lumicolchicine (an isomer of colchicine, without tubulin-binding property) may shed additional, and necessary, light on the discussed problems. From another side, the effect(s), if any, of phenobarbital should be tested on arterial SMC.

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Presence of digoxin detectable by radioimmunoassay in *Tetrahymena*

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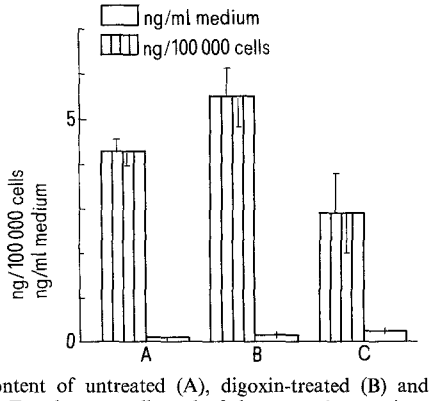
Summary. Digoxin was demonstrated in *Tetrahymena pyriformis* by radioimmunoassay, at a concentration of 4.3 ng/100,000 cells. Pretreatment of the cells with digoxin or ouabain did not significantly alter the digoxin concentration of the progeny generations.
Key words. *Tetrahymena;* digoxin.

The unicellular ciliate protozoon *Tetrahymena* either possesses receptors for the hormones of higher organisms, or is able to form[2,3] binding sites in the presence of the hormones. It binds insulin, thyroxine (and its precursors), thyrotropin, gonadotropins, etc., and is frequently also capable of a specific response to these. Although as a unicellular organism it represents a very low phylogenetic level it is a highly differentiated organism of its kind, and itself contains several hormones, such as serotonin[4], epinephrine[5], insulin[6], somatostatin[7], beta-endorphine[8], and even ACTH-like molecules[9] and relaxin[10]. Our earlier experimental observations[11] have suggested that apart from these hormones digoxin, a plant glycoside of steroid structure, could occur in *Tetrahymena*. A detailed investigation of this is reported in this paper.

Tetrahymena pyriformis GL cells, maintained in 0.1% yeast extract containing 1% Bacto-tryptone medium at 28 °C, were used after 2 days of culturing. Part of the cultures was not treated to serve as control, part was transferred to a medium containing 12.25 µg/ml digoxin (USP XIX) or 50 µg/ml ouabain (Fluka, Switzerland). The control cultures were transferred to the plain medium for 24 h, and were centrifuged to separate the cells from the medium. The cells were disrupted by sonication, and both sediment and supernatant were assayed for digoxin content with the Digoxin RIA kit (Amersham, England). The experimental cultures were incubated in presence of digoxin or ouabain for 24 h, after which they were returned to plain medium, which was exchanged several times during a further 5-day period of incubation. This was followed by centrifugation, sonication, and digoxin assay, as above. 10 tube cultures were set up for each assay; contamination of the glassware used with digoxin could be ruled out with certainity.

The digoxin content of the culture fluid was within the range of experimental error (0.1–0.25 ng/ml), thus the medium itself contained only traces of digoxin if any. The digoxin content of



Digoxin content of untreated (A), digoxin-treated (B) and ouabain-treated (C) *Tetrahymena* cells and of the respective nutrient media. S_x values were indicated by the thin lines.

the control cells – which had not been previously exposed to exogenous digoxine – was 4.3 ng/100,000 cells (fig.). Taking into consideration that the therapeutic dose level of digoxin is 0.8–4.5 ng/ml of sera for humans, the concentration of digoxin or immunologically demonstrable digoxin-like materials (the kit used also detects digitoxin and dihydro-digoxin) in *Tetrahymena* is appreciable.

We performed pretreatment with digoxin or the related glycoside ouabain on the ground that the glycosides may stimulate their own synthesis under certain conditions[11]. The quantitative relations of uptake differed considerably between digoxin- or ouabaine-pretreated and control cells, but statistical evaluation was hampered by extreme intraassay variations. The marked, although not significant increase in the digoxin content of the digoxin-pretreated cells might have been due to intracellular retention of part of the considerable amount of digoxin used for preexposure. This could be ruled out in the case of ouabain, since the cross reaction of the kit used with ouabain was only 0.029% of its reaction with digoxin, i.e. it did not appreciably differ from the control.

The precise role of endogenous digoxin for *Tetrahymena* is at the time being obscure, but so are the role of ACTH, insulin, somatostatin and relaxin. The authors[6–10] who detected the latter hormones in *Tetrahymena* have postulated that after release from the cell, the hormones are involved in the control of receptor activity by a feedback mechanism. We failed to find evidence of this in earlier studies[12] or in the present experiments. Digoxin does not occur in man, except when administered as a drug, and its natural occurrence in other animals is exceedingly rare (e.g. in toad poison)[13] – what might then be its biological role in *Tetrahymena*? It seems very likely that the lowest levels of phylogenesis represent an 'experimental stage' in respect of the biosynthesis of polypeptides and steroids (like tetrahymanol) and the byproducts or intermediary products of such biosynthetic processes could result in the formation of hormones and of digoxin-like molecules as well.

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Somatic variation in micropropagated clones of *Cichorium intybus*

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Summary. An experiment aiming to establish whether temporary or persistet somatic variation can arise in clones obtained in vitro by micropropagation has been performed on *Cichorium intybus*. The results point out that persistent modifications of the phenotype can be observed after six cycles of cloning and that when the length of the cloning period is varied these appear to be differential responses of the same genotype to the micropropagation.
Key words. Cichorium intybus; clones, micropropagated; somatic variation.

A number of authors have clearly established that, in vegetatively propagated plants, differences in a given character may occur between the propagules of the clone. Such variations may arise during the first asexual propagation and then disappear after a period of time, as is observed with root characteristics of *Populus deltoides*[1] and in tiller number of *Lolium perenne*[2]. These temporary effects or 'c' effects, are generally ascribed to physiological or morphological influences arising from the specific environment in which the original plant developed[3]. On the other hand it has been observed that the above-mentioned variations may be persistent over further cycles of propagation[4,5] and Breese et al.[6], who found persistent variations in rates of tillering in *Lolium perenne*, inferred that intraclonal differences may be due to cytoplasmic or plasmon heterogeneities originated during the initial sub-cloning.

Though the nature of these extranuclear differences is not always easy to interpret, some authors[7–9] suggested that in asexually reproduced organisms differences could occur as result of degeneration of the cytoplasm during ageing.

The present investigation aims to establish whether temporary of persistent somatic variation can also arise in clones obtained in vitro by micropropagation, and whether the expression of several characters in one genotype can be altered by varying the lenght of the cloning period.

Materials and methods. Seeds of *Cichorium intybus* cultivar Chioggia were surface-sterilized by 30 min of treatment with 20% sodium hypochlorite.

They were then washed with sterile distilled water and placed on a medium with 3% sucrose solidified with 0.5% agar.

One week after germination the primary roots of six seedling were excised and discarded while the shoot tips were transferred to a medium stimulating the induction of adventitious shoots (modified Murasige and Skoog[10] which contained 0.3 mg/l GA, 0.01 mg/l NAA and 0.2 mg/l BAP) obtaining a population of ramets from each seed.

From these initial populations two types of clones were established differing from each other in the length of their propagation cycles, that is, one type was cloned every 14 days (short