ment including temperature, humidity, percentage of CO_2 in the atmosphere, number of cells in the inoculum and time of harvesting. Also, differences in batches of fetal calf serum were avoided by preparing the complete medium in advance and freezing it down (−70 °C) in aliquots.

Furthermore, leakiness of the membranes due to the penetration of the cell is a frequent cause of lowered MRP[8]. In our experiments this possibility was apparently excluded since repeating or prolonging the penetration of the cell yielded reproducible results, showing that cells survive the treatment and that the MRP values recorded can be considered as real. The same suggestion can be made on the basis of the trypan blue test which showed no difference in viability before and after MRP measurements. It has to be pointed out, however, that exclusion of trypan blue is indicative of cell death but is only of limited value for testing the integrity of the cells.

As for the electrophysiological measurements, low resistance electrodes were used throughout our experiments. Although high resistance electrodes are known to cause less damage to the cell membrane, we prefer to use low resistance electrodes as they yield less artifactual measurements due to relatively lower and more stable tip potentials[9].

Finally, one should consider the possibility that the more accessible larger cells exhibit higher MRP values than the smaller cells which are more difficult to penetrate[9]. In our experiments, care was taken to avoid this selection by excluding arbitrarily both the very large and very small cells. Accordingly, it was concluded that the MRP values obtained represent real values. The MRP value for confluently grown cells is significantly higher than for cells in the subconfluent state. Logarithmically growing cells show in our experiments a higher MRP value as compared to the subconfluent cells, although this difference is nonsignificant. A similar phenomenon has been described by others[10] and is thought to be due to the initiation of the cell cycle.

The differentiation-induced increase in MRP probably reflects a true functional alteration of the tumor cell membrane.

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Colchicine-induced appearance (proliferation) of smooth sarcoplasmic reticulum in arterial smooth muscle cells

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Summary. Colchicine treatment resulted in the appearance and proliferation of smooth sarcoplasmic reticulum in some smooth muscle cells of the aortic and pulmonary trunk walls in the rabbit. The significance of cytoplasmic microtubules and/or membrane-bound tubulin for the morphogenesis, functioning and control of smooth endoplasmic reticulum in different kinds of cells is discussed.

Key words. Rabbit aorta; smooth muscle cells, arterial; sarcoplasmic reticulum; colchicine; tubulin; microtubules.

We have previously reported that treatment with a microtubule-disassembling agent, colchicine, resulted in an accumulation of secretion granules[1,2] and lysosomes[3], and vacuolar dilation of rough endoplasmic reticulum[1,2], in smooth muscle cells (SMC) from rabbit aortic arch, thoracic aorta and pulmonary trunk. During the course of these studies, we found that some but not all of the SMC responded with an evident increase in content of the smooth sarcoplasmic reticulum (SSR), which, from the ultrastructural point of view, resembled the well-known phenobarbital-induced proliferation of the smooth endoplasmic reticulum in hepatocytes. We think that this effect of colchicine, previously undescribed, may not only be of interest for our understanding of arterial SMC, but may also be of more general importance for several reasons. Firstly, because cytoplasmic microtubules and/or membrane-bound tubulin may be involved in the functioning and control of 1) contraction-relaxation cycle of SMC[4], and 2) microsomal drug-metabolizing enzymes in hepatocytes[5,6], and secondly because phenobarbital may share some microtubule-disassembling properties[7]. Moreover, the observation of this effect raises the intriguing question of whether some microtubule-disassembling agents are able to induce alteration(s) in the function of the smooth endoplasmic reticulum in different kinds of cells (SMC, hepatocytes, steroidogenic cells, skeletal muscle cells, cardiomyocytes, etc.).

Materials and methods. Colchicine (0.125 mg/100 g b.wt) was injected i.p., and 4 h later the animals were sacrificed. Untreated animals were used for controls. Longitudinal strips were taken from aortic arch, thoracic aorta and pulmonary trunks of rabbits aged 20 days, both from control and colchicine-treated groups. The strips were immediately immersed in cold 3% glutaraldehyde and postfixed in cold 1% osmium tetroxide, both in 0.1 M phosphate buffer, pH 7.4. Dehydration was in alcohols and acetone, and embedding in Durcupan ACM (Fluka). Ultrathin sections were stained with uranyl acetate and lead citrate. A JEM 7A electron microscope was used.

Results and discussion. The SMC from control rabbits revealed well-developed rough endoplasmic reticulum, Golgi complex and numerous microtubules (fig. 1). Usually, a part of the microtubules is spatially associated with rough endoplasmic reticulum. Briefly, these SMC were in secretory phenotype[2,8] according to the current knowledge about the arterial SMC phenotype expression[9].

Colchicine treatment resulted in the appearance, and proliferation, of SSR elements, both of tubulo-vesicular (figs 2 and 3) and cisternal (fig. 4) nature, in some of the SMC studied. Dilation of rough endoplasmic reticulum was, however, characteristic for a greater population of SMC[1,2,8]. Figure 2 shows a situation resembling the phenobarbital-induced smooth endoplasmic reticulum proliferation in hepatocytes thus suggesting that both agents, colchicine and phenobarbital, may share some common mode of action in respect to their effects on endoplasmic reticulum, though it is of course possible that these two agents may lead to a similar morphological result via different mechanisms of action. Nevertheless, the colchicine effect described in the present report raises some intriguing questions. What is the significance of the various cytoskeletal ele-

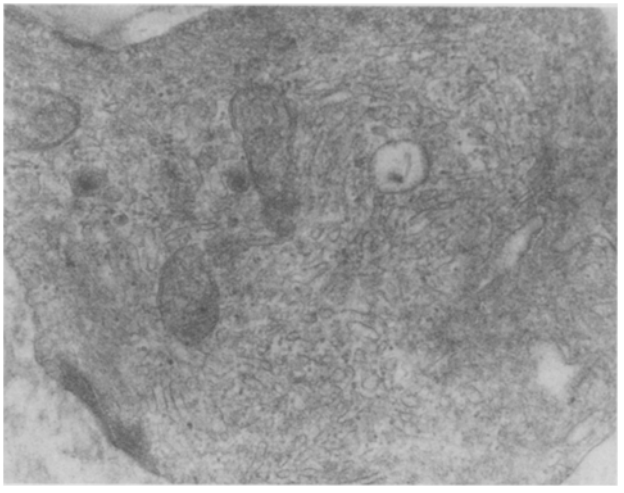

Figure 2. SMC from colchicine-treated group. The appearance and proliferation of SSR elements of tubular type are evident. This SMC contained no microtubules. ×20,000.

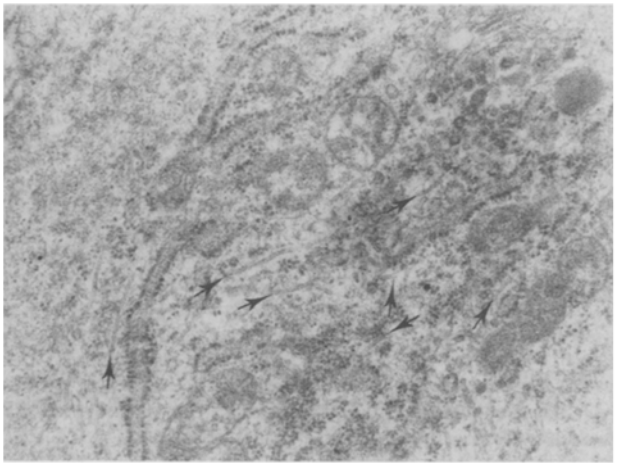

Figure 1. SMC from control group. Well-developed rough endoplasmic reticulum and Golgi complex. The microtubules (arrows) associated with cisternae of rough endoplasmic reticulum are noted. ×20,000.

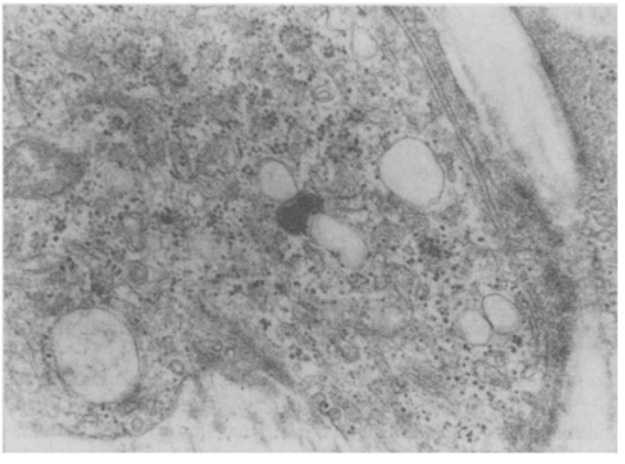

Figure 3. SMC from colchicine-treated group. The appearance of SSR elements of tubulo-vesicular type is demonstrated. This SMC contained no microtubules. ×20,000.

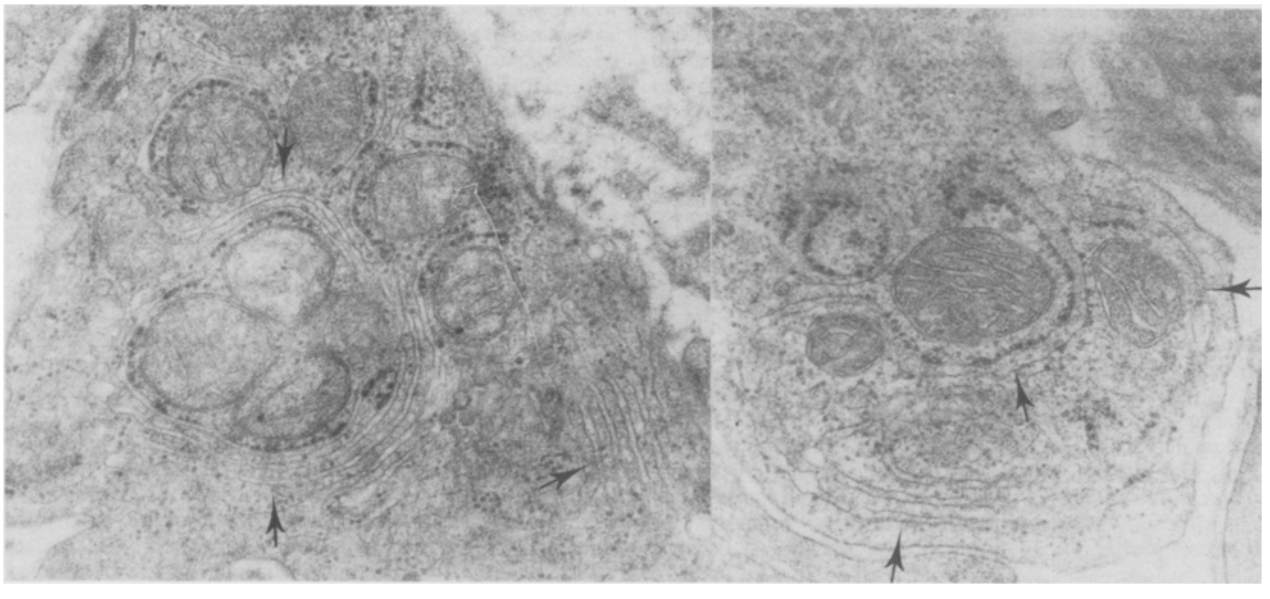

Figure 4. SMC from colchicine-treated group. Numerous cisternae of SSR (arrows) are noted. Some of them are located near the mitohondria. Only the cisternal surface facing the mitochondria, preserves many attached ribosomes, which may suggest that SSR cisternae, via a progressive detachment of ribosomes from the cisternae of rough endoplasmic reticulum, can appear in SMC when cytoplasmic microtubules are disrupted[10]. ×20,000.

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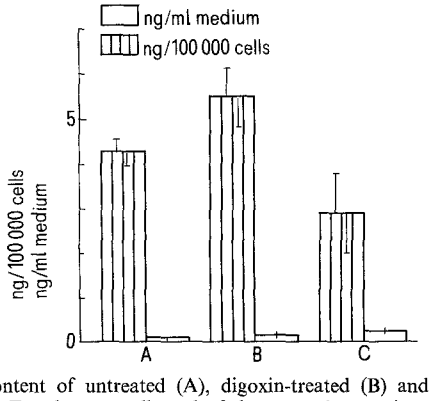
Summary. Digoxin was demonstrated in *Tetrahymena pyriformis* by radioimmunoassay, at a concentration of 4.3 ng/100,000 cells. Pretreatment of the cells with digoxin or ouabain did not significantly alter the digoxin concentration of the progeny generations.
Key words. *Tetrahymena;* digoxin.

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.