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Membranes of *Tetrahymena*

IV. Isolation and Characterization of Temperature-Responsive Smooth and Rough Microsomal Subfractions

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Summary. Temperature-responsive microsomes of the ciliate protozoan *Tetrahymena* have been originally fractionated by step centrifugation on two-layered, Mg^{2+}-containing sucrose gradients. Three fractions have been obtained, which are termed smooth I, smooth II and rough according to the appearance of the membrane vesicles upon electron-microscopy. Smooth I, smooth II, and rough microsomes exhibit RNA/protein ratios of 0.09, 0.20, and 0.34; their phospholipid/protein ratios and their neutral lipid/phospholipid ratios were 0.52, 0.43 and 0.25, and 0.17, 0.18 and 0.13, respectively. All three fractions contain equivalent, low succinic dehydrogenase and 5′-nucleotidase activities. Glucose-6-phosphatase and acid phosphatase are more concentrated in smooth I membranes than in rough membranes. The reverse is true for ATPase. The smooth II membranes occupy an intermediate position except that their ATPase activity is the lowest of the three fractions. The specific activities of these enzymes of the three microsomal fractions are compared to those of homogenates of whole cells. Thin-layer chromatography reveals a very similar polar and nonpolar lipid pattern of the three microsomal fractions. The major phospholipid compounds are phosphatidylethanolamine, glycerideaminoethylphosphonate and phosphatidylcholine, while diglycerides, an unknown NL-compound, and triglycerides are the major apolar lipids. Gas liquid chromatography shows that the fatty acids are mainly even-numbered ranging between C_{12} and C_{18}. The smooth I, smooth II and rough membranes contain 65.2, 69.3 and 72.7% unsaturated fatty acids in their polar lipids, whereas only 52.7, 49.7 and 48.3% unsaturated acids are found in their apolar lipids, respectively. The fatty acids are more unevenly distributed among the individual polar lipids than in the apolar ones.

The unicellular protozoan, *Tetrahymena,* constitutes an attractive system for studies on the structure, function and biogenesis of membranes [14, 20–22, 25–27, 31, 41]. *Tetrahymena* is a phylogenetically simple eukaryotic cell, which contains the various types of intracellular membranes, e.g. those of mitochondria, peroxisomes, nuclei, smooth and rough endoplasmic reticulum, which are also found in far more complex eukaryotes such as mammalian cells. The only exception is that *Tetrahymena* normally possess no typical Golgi apparatus [for review *see* 7, 11].

Most interestingly, all of the intracellular membranes of *Tetrahymena* can undergo reversible thermotropic transitions which have recently been visualized directly in vivo by freeze-etch electron-microscopy. Specifically, areas depleted of intramembranous particles separate laterally from particle-bearing areas on the membrane fracture faces [29, 38, 39, 42]. However, for a more detailed understanding of these reversible thermotropic membrane reorganizations it is primarily necessary to isolate membranes from *Tetrahymena,* and to analyze their constituents, the lipids in particular. In any case, the isolated membranes must retain their thermotropic character, need to be obtained in satisfactory yield, and should be smooth, i.e. their surfaces should not be studded with ribosomes, for example, which could injure studies on the membrane surfaces. These criteria are met in particular by the light smooth microsomal membranes which we originally separate from the heavy smooth and rough microsomal membranes of *Tetrahymena.*

Materials and Methods

Cultures

Axenic cultures of *Tetrahymena pyriformis* (amicronucleate strain GL) were statically grown at 28 °C in the logarithmic phase (~50,000 cells/ml) in a medium consisting of 0.75% proteose peptone, 0.75% yeast extract, 1.5% glucose, 0.1 mM ferric citrate, 0.05 mM $CaCl_2$ and 1 mM $MgSO_4$ [18]. Cell counts were performed in a Fuchs-Rosenthal-counting-chamber.

Isolation and Subfractionation of Microsomes

The cells were harvested at 4 °C by centrifugation at $10{,}000 \times g_{av}$ min. *Tetrahymena* microsomes were isolated and subfractionated, respectively, according to a modified method of Dallner [2, 4]. The cells were suspended in 0.8 M sucrose, 5 mM Tris-HCl, 5 mM $MgCl_2$ (pH 7.2) at 0–4 °C. After 2 min, the concentration of sucrose in this suspension was reduced to 0.08 M by adding sucrose-free buffer. The cells were then immediately centrifuged at $15{,}000 \times g_{av}$ min (~4 °C). The cell pellet resuspended in buffered 0.4 M sucrose was homogenized by hand using a Potter-Elvejhem grinder with a teflon pestle at 0–4 °C. Cell rupture was monitored by phase-contrast microscopy and grinding continued until about 90–95% of the cells were disrupted. The homogenate was then centrifuged twice at $100{,}000 \times g_{av}$ min (~4 °C). Fifteen ml of the postmitochondrial supernatant was layered over 10 ml of buffered 1.33 M sucrose. After centrifugation at $2 \times 10^7 \times g_{av}$ min (~4 °C; Weinkauf ultracentrifuge, rotor 8 × 38) the upper 0.4 M sucrose layer was discarded. The discrete white layer slightly below the 0.4–1.33 M sucrose boundary yields fraction I. The rest of the 1.33 M sucrose yields fraction II, with the exception of the last few drops. The latter together with the pellet yield the third fraction. Buffered 0.4 M sucrose was added to the three fractions and centrifuged at $6.6 \times 10^6 \times g_{av}$ min (~4 °C). The pellets were then resuspended in 5 mM Tris-HCl (pH 7.2) and repelleted at $6.6 \times 10^6 \times g_{av}$ min (~4 °C).

Electron-microscopy

Microsomal pellets were fixed with 1% glutaraldehyde in 0.05 M Na cacodylate (pH 7.2) at 0–4 °C for 10 min and postfixed with 2% OsO_4 buffered with cacodylate at this temperature for 1.5 hr before they were dehydrated step-wise with increasing proportions of ethanol to propylene oxide and embedded in Epon 812. Thin sections were cut on a Reichert OmU_2 ultramicrotome, double-stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmiskop Ia.

Chemical Determinations

The total lipids of whole cells and the three microsomal fractions precipitated with iced 5% trichloroacetic acid (TCA) were extracted with chloroform-methanol (2:1) according to Folch *et al.* [8]. The organic phases were evaporated, freeze-dried and weighed, before we determined from aliquots the phospholipid content according to Gerlach and Deuticke [9]. The phospholipid amount was subtracted from the total freeze-dried material thus yielding the total neutral lipid content. From the chloroform/methanol extracted TCA-precipitates, the total RNA-content was determined according to the orcinol-method [23] and the total protein content according to Lowry *et al.* [19], using bovine serum albumin as a standard.

Enzyme Assays

Glucose-6-phosphatase (E.C. 3.1.3.9) was determined at V_{max} as described by Baginski *et al.* [1]. When the temperature dependence of this enzyme was tested, the incubation temperatures were varied between 28° and 7 °C. The kinetics of the phosphate release were linear at 7° and 28 °C. *5'-nucleotidase* (E.C. 3.1.3.5) was assayed as in Gerlach and Hiby [10]. *Acid phosphatase* (E.C. 3.1.3.2) was measured as described in Walter and Schmitt [37]. *Succinic dehydrogenase* (E.C. 1.3.99.1) was assayed as in King [15]. *ATPase* (E.C. 3.6.1.3) was determined as follows: A solution containing 5.5 mM $MgCl_2$, 11 mM KCl, 5.5 mM ATP, 0.8% mercaptoethanol, 0.3 M Tris-HCl (pH 7.4) was warmed for 10 min at 37 °C; then the samples (~0.15 mg protein) were added and incubated for a further 10 min. Released inorganic phosphate was determined as in [1].

Lipid Analysis

Polar Lipids. Aliquots of the chloroform-dissolved lipid fractions were applied to 20 × 20 cm plates, coated with silica gel H (Merck, Darmstadt) and preactivated at 110 °C for 1 hr. These plates were two-dimensionally developed [16]: The solvent systems were chloroform/methanol/water/acetic acid (50:20:0.8:1.5; v/v/v/v) and chloroform/methanol/7 M NH_4OH (50:20:0.3; v/v/v) in the first and second dimension, respectively. Visualization of the individual polar lipids was achieved by spraying the chromatograms with 0.05% Rhodamine B in ethanol-supplemented 5% Tinopal [24]. The visualized spots were scraped out, and phosphorus was determined as above. All spots contained phosphorus with the exception of a minor one (separating occasionally into two), which contained sugar [12]. Further identification of the spots was performed by spraying the developed chromatograms with ninhydrin and by co-chromatography with reference substances purchased from Applied

Science Lab. (State College, Pa.) and Serva (Heidelberg). The main spot containing phosphatidylethanolamine and its phosphonoanalogue glycerideaminoethylphosphonate was separated by re-chromatography on silica gel G plates (Merck). Development was with chloroform/acetic acid/methanol/water (75:25:5:0.5; v/v/v/v). Two minor spots, which contained phosphorus and showed ninhydrin-positive reaction were tentatively identified as ceramideaminoethylphosphonate and ceramidemonomethylaminoethylphosphonate [35, 36].

Nonpolar Lipids. These were separated on 20 × 20 cm plates coated with a 3:1 (v/v) mixture of silica gel H (Merck)—cellulose (Macherey, Nagel and Co., Düren, West Germany). Development was with petroleum/ether/chloroform/acetone (50:20:3; v/v/v) and visualization was with tinopal as above.

Fatty acids. Saponification, methylation, and gas chromatography of the fatty acids were performed as described earlier [42]. The fatty acid methyl esters were identified by comparison with retention times of known standards (Serva). For further identification of the unsaturated fatty acid methyl esters, these were gas-chromatographically compared before and after hydrogenation on a platinum oxide catalyst. One of the two tetradecanoic acids as well as the two octadecadienoic acids were tentatively identified as 14:0 iso, 18:2 Δ 6,9 and 18:2 Δ 9,12, respectively. Quantitative determination of the fatty acids was performed according to the method of Duncombe [5], with elaidic acid as a standard.

Results

Micromorphology

Only smooth-surfaced membranes about 7–8 nm thick are revealed in the microsomal fraction (Fig. 1), which we term therefore smooth I. The smooth I membranes form vesicles the diameter of which range from 40 nm up to 1,000 nm (average: ~200 nm). Again exclusively smooth, 7–8 nm thick membranes are detected in fraction II (Fig. 2), thus designated as smooth II. The diameter of the vesicles range from 40 nm to 500 nm, with an average of about 150 nm. Not infrequently, the vesicles contain high electron dense material. Such vesicles can also be found relatively often in intact *Tetrahymena* cells [for review *see* 7, 11]. Many free ribosomes and ribosomal aggregates co-sediment with the smooth II fraction. The pellet fraction contains mainly clumped ribosomes. The membranous portion of this fraction reveals ribosome studded cisternae and vesicles reaching a diameter of up to 350 nm (Fig. 3). This fraction is therefore designated as rough. Moreover, glycogen granules distribute regularly throughout this fraction, besides occasional disaggregated cilia axonemes, coated vesicles, as well as smooth vesicles with a moderate electron dense stain (probably premucocysts). It is noteworthy that structurally intact mitochondria or peroxisomes could not be detected in any of the three microsomal subfractions.

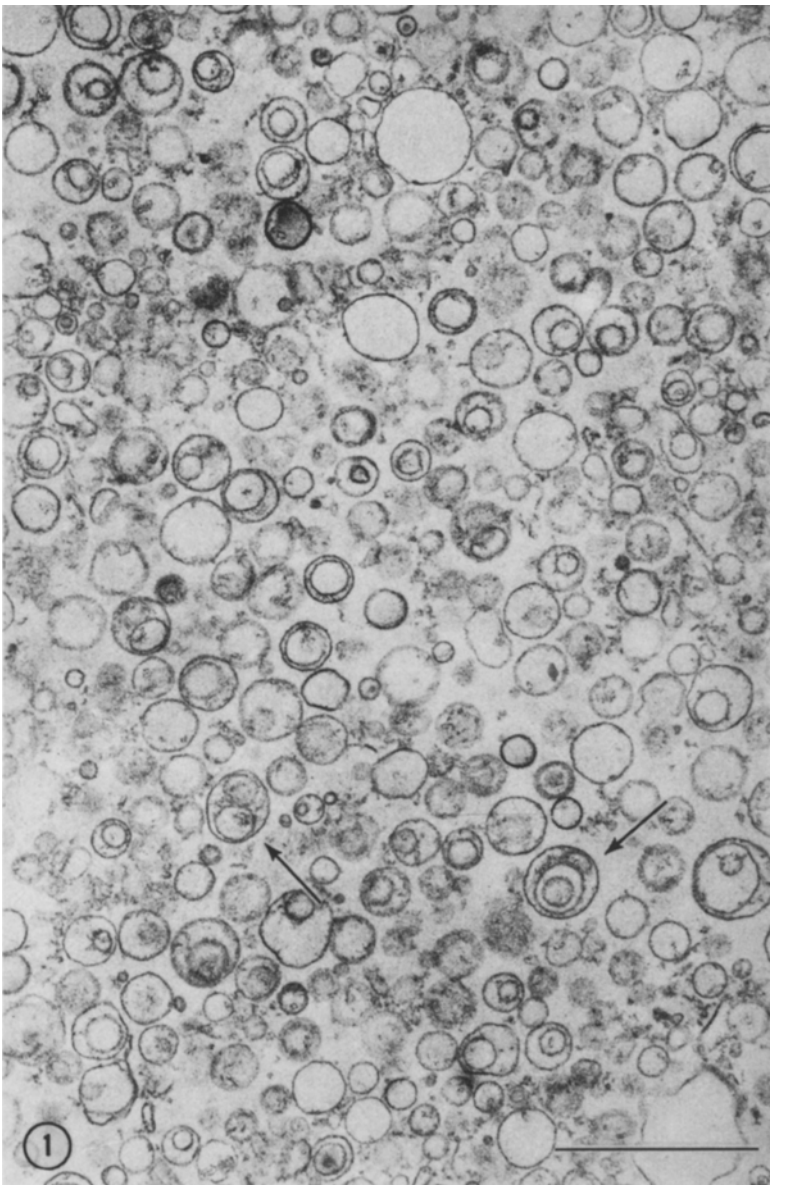

Fig. 1. Survey thin-sectioning electron-micrograph of the smooth I microsomes showing only smooth-surfaced membrane vesicles. Besides unilamellated vesicles, double membrane vesicles and occasional trilamellar vesicles (arrows) can also be observed. Note the absence of ribosomes! Bar represents 1 μm. × 27,000

Yield and Gross Composition

The content of RNA, protein, phospholipid and neutral lipid of whole *Tetrahymena* cells and the three microsomal membrane fractions is given

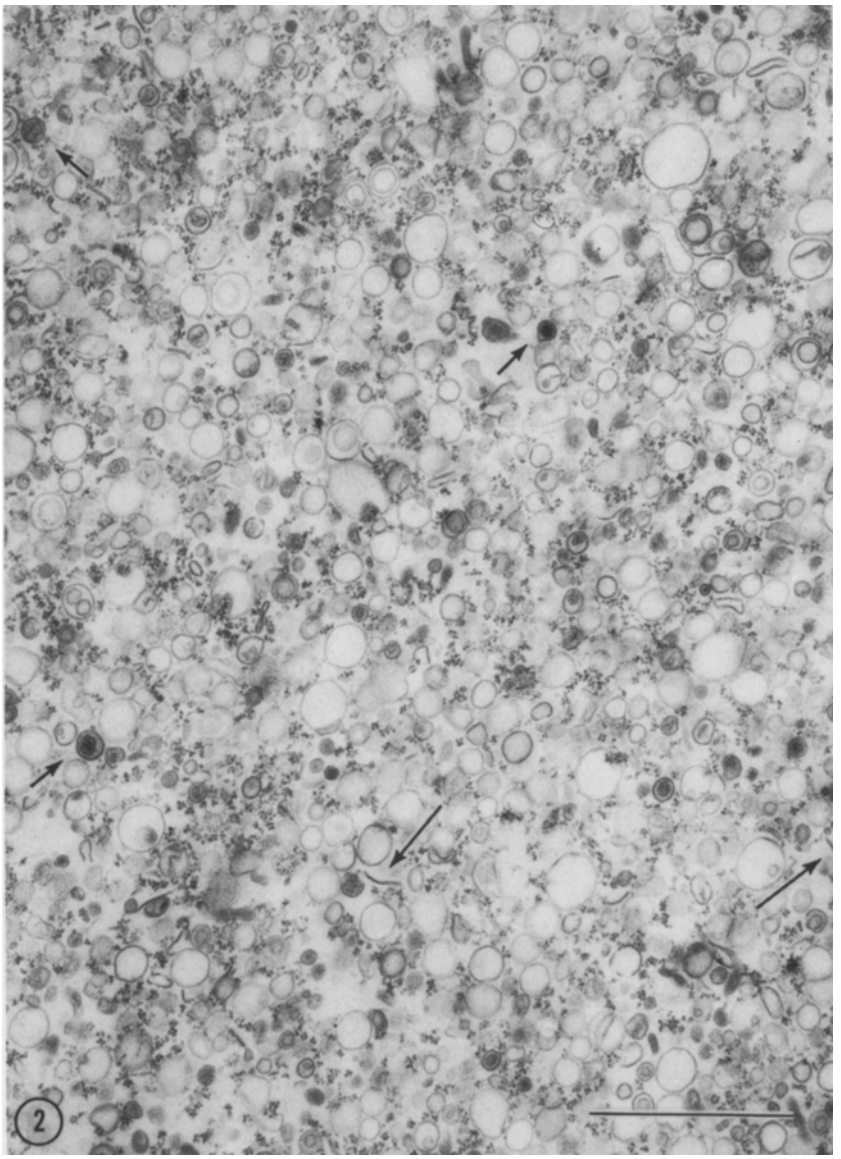

Fig. 2. Survey thin-sectioning electron-micrograph of the smooth II microsomes. The smooth-surfaced membrane vesicles are contaminated with many co-sedimenting nonmembrane-bound ribosomal aggregates. Long arrows: open-end membrane sheets. Short arrows: vesicles containing electron-dense material. Bar represents 1 μm. ×27,000

in Table 1. We can calculate that 0.8, 1.8 and 7.1% of the total cell RNA, 1.2, 1.3 and 3% of total protein, 3.4, 3.0 and 3.6% of total phospholipid, and 1.7, 1.6 and 1.3% of total neutral lipid is recovered in the

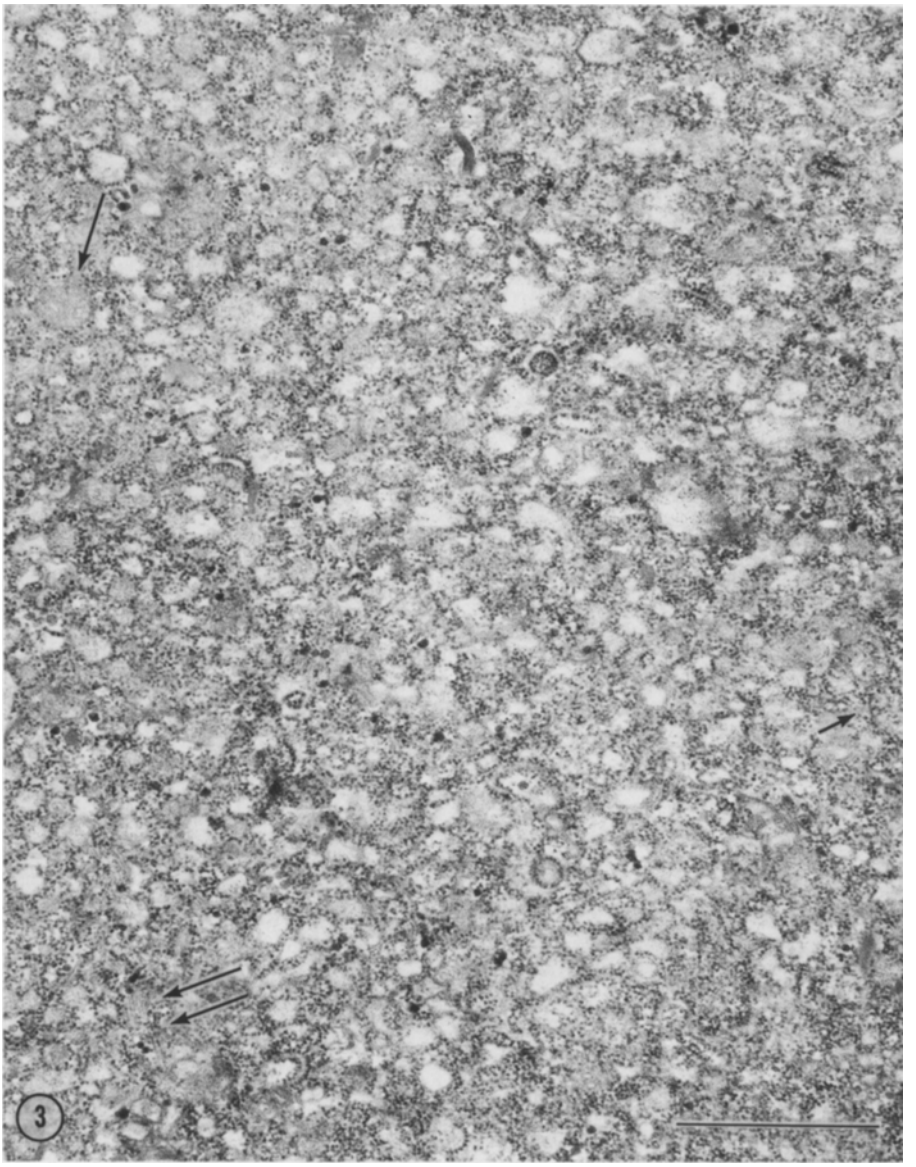

Fig. 3. Survey thin-sectioning electron-micrograph of the rough microsomes. These mainly contain a lot of ribosomes and ribosome-studded vesicles. Glycogen granules distribute also regularly throughout this fraction. Double arrow: disrupted cilia axonemes. Short arrow: coated vesicle. Long arrow: smooth vesicles with moderate electron-dense stain. Bar represents 1 μm. ×27,000

smooth I, smooth II and rough microsomes, respectively. The rough microsomes display a phospholipid/protein ratio of 0.25, while the smooth I and smooth II membranes have ratios of 0.52 and 0.43, respectively. In

Table 1. Gross composition of *Tetrahymena* cells and microsomal subfractions[a]

	Whole cells	Microsomal subfractions		
		Smooth I	Smooth II	Rough
RNA[b]	243	2.0	4.4	17.1
Protein[b]	1800	22.2	24.1	50.4
Phospholipid[b]	346	11.8	10.4	12.5
Neutral lipid[b]	121	2.0	1.9	1.6
RNA/protein[c]	0.14	0.09	0.20	0.34
Phospholipid/protein[c]	0.19	0.52	0.43	0.25
Neutral lipid/phospholipid[c]	0.35	0.17	0.18	0.13

[a] Means of three experiments.
[b] pg/cell.
[c] w/w.

accord with the electron-microscopic data, the lowest RNA/protein ratio (0.09) is found in the smooth I fraction, while the smooth II and rough fractions display ratios of 0.20 and 0.34, respectively. The ratio of neutral lipid/phospholipid is very similar in the two smooth microsomal membrane fractions ($\sim$0.18), while it is somewhat lower in the rough membranes (0.13).

Enzymes

Table 2 represents the specific activities of some enzymes associated with whole cells and the three microsomal fractions. Among the latter the succinic dehydrogenase and the 5′-nucleotidase reveal approximately equal specific activities. The other tested enzymes distribute unevenly, as is also known in other cells such as e.g. *Acanthamoeba* [34] and mammalian liver [e.g. 2, 3, 30]. Thus, the smooth I membranes display the highest activity of the glucose-6-phosphatase and the acid phosphatase. In contrast, the ATPase is more active in the rough microsomes. Conspicuously, the smooth II membranes reveal enzyme activities which are always intermediate between the rough and smooth I microsomes. It is noteworthy that, in *Acanthamoeba* and mammalian liver, the smooth microsomes display a relatively lower glucose-6-phosphatase activity, but higher activities of ATPase than the rough microsomes [4, 30, 34], which contrasts to *Tetrahymena*. Whether these differences reflect some other functional specifities of the smooth and rough microsomes of *Tetrahymena* or whether they are due to the different isolation procedure used cannot be decided at present.

Table 2. Enzymes associated with *Tetrahymena* cells and microsomal subfractions[a] (Relative specific activity = specific activity of the fraction/specific activity of whole cells.)

Enzymes	Microsomal fractions					
	Smooth I		Smooth II		Rough	
	Spec. act.	Rel. spec. act.	Spec. act.	Rel. spec. act.	Spec. act.	Rel. spec. act.
G 6 Pase[b]	0.080	4.44	0.062	3.45	0.064	3.55
ATPase[b]	0.125	1.52	0.086	1.05	0.153	1.87
Acid Pase[b]	0.090	0.41	0.053	0.24	0.042	0.19
5′-nucleotidase[b]	0.016	0.19	0.016	0.19	0.012	0.14
Succ. dehydrogenase[b]	0.003	0.14	0.003	0.14	0.004	0.18

[a] Means of four experiments.
[b] μmoles/min/mg protein.

Polar Lipids

The quantitative composition of the two-dimensionally separated phospholipids (Fig. 4) from *Tetrahymena* cells as well as from the microsomal membranes can be seen in Table 3. The major phospholipids are phosphatidylethanolamine, glycerideaminoethylphosphonate, and phosphatidylcholine. A very similar phospholipid pattern is found among the three microsomal subfractions (Table 3). However, the microsomes contain less phosphatidylethanolamine, cardiolipin, and more glycerideaminoethylphosphonate, ceramideaminoethylphosphonate and lysophosphatidylcholine than whole cells.

The fatty acid residues of the phospholipids are mainly even-numbered ranging from C_{12} to C_{18} in all three microsomal fractions (Table 4). Remarkably, approximately half of the total fatty acids is constituted by equal portions of the two octadecadienoic acids and the octadecatrienoic acid (Table 4). Moreover, the total phospholipids of the three microsomal subfractions contain different amounts of unsaturated fatty acids (Table 4). Thus, the smooth I microsomes contain only 65.2% unsaturated acids, while 69.3% are detected in the smooth II membranes. The rough microsomes possess the highest percentage of unsaturated acids (72.7%). Moreover, the percentage of unsaturated fatty acids is uneven among the individual phospholipids (Table 5).

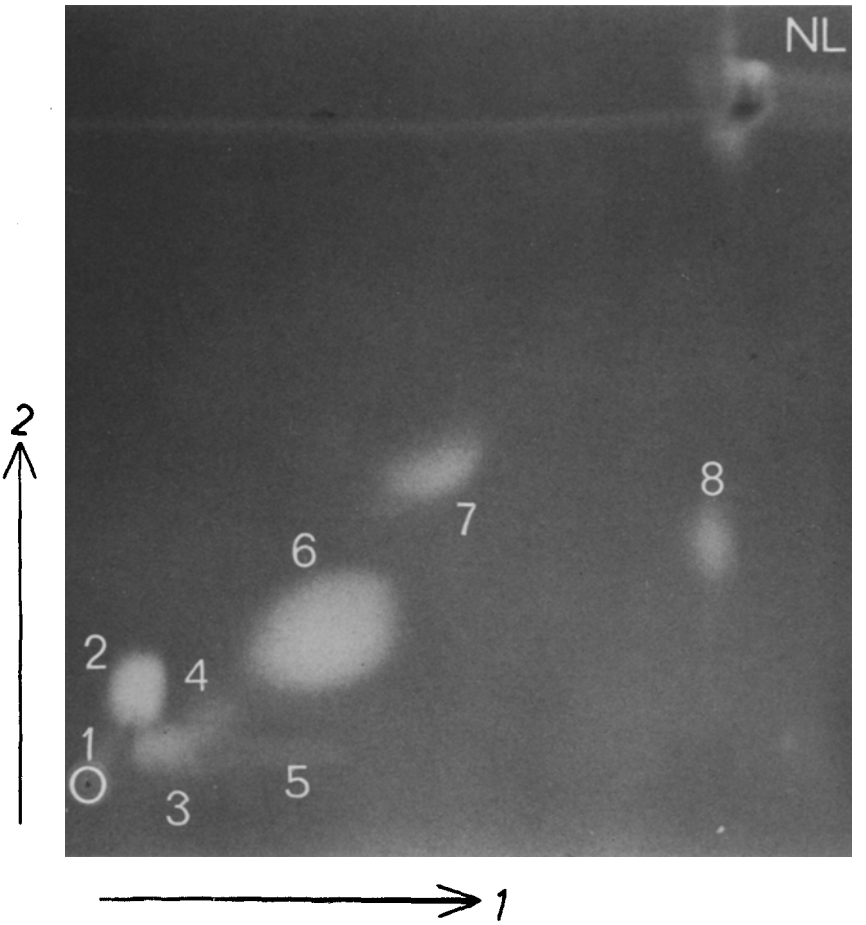

Fig. 4. Two-dimensional thin-layer chromatogram of the polar lipids of *Tetrahymena* cells, sprayed with tinopal and photographed at 254 nm. Arrows indicate first and second dimension. O, origin; 1, lysophosphatidylcholine; 2, phosphatidylcholine; 3, ceramideaminoethylphosphonate; 4, ceramidemonomethylaminoethylphosphonate; 5, phosphatidylinositol; 6, phosphatidylethanolamine and glycerideaminoethylphosphonate; 7, unknown glycolipid; 8, cardiolipin; NL, neutral lipids

Nonpolar Lipids

These separate on the chromatograms mainly into three spots (Fig. 5) containing (a) diglycerides (this spot contains probably also monoglycerides and free fatty acids), (b) an unknown NL-compound with saponifiable fatty acids [*see also* 17] and (c) triglycerides. The front contains some unidentified fatty acid esters. Tetrahymanol contributes only traces to

Table 3. Phospholipid composition of whole cells and microsomal subfractions of *Tetrahymena* in mole % of total lipid phosphorus[a]

Phospholipid[b]	Whole cells	Microsomal subfractions		
		Smooth I	Smooth II	Rough
PE	35.0	30.0	29.6	30.2
GAEP	19.0	23.0	21.6	22.4
PC	18.0	18.1	18.5	16.9
LPC	1.9	4.8	4.7	4.8
CAEP	11.3	14.2	14.3	13.7
MMAEP	4.1	4.7	4.3	5.1
PI	4.2	3.9	5.1	4.7
CL	6.5	1.3	1.9	2.2

[a] Average of three experiments.
[b] PE, phosphatidylethanolamine; GAEP, glycerideaminoethylphosphonate; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; CAEP, ceramideaminoethylphosphonate; MMAEP, ceramidemonomethylaminoethylphosphonate; PI, phosphatidylinositol; CL, cardiolipin.

Table 4. Major fatty acid composition of total lipids (TL), polar lipids (PL) and neutral lipids (NL) from whole cells and microsomal subfractions of *Tetrahymena* (% of total fatty acids)[a]

Fatty acids	Whole cells			Microsomal subfractions								
				Smooth I			Smooth II			Rough		
	TL	PL	NL	TL	PL	NL	TL	PL	NL	TL	PL	NL
12:0	2.5	2.3	2.8	2.8	2.9	3.6	2.4	2.5	4.4	2.0	3.0	3.4
14:0 iso	8.8	7.0	10.6	10.7	10.3	11.2	9.5	9.6	10.1	9.7	8.2	10.6
14:0	3.1	2.3	4.3	3.2	3.4	4.4	3.0	3.0	5.1	2.9	2.9	4.5
15:0	1.0	1.0	2.2	1.2	1.6	3.7	1.2	1.0	4.2	1.1	1.0	3.2
16:0	9.2	8.1	12.0	11.9	12.0	12.8	11.7	9.5	14.6	10.9	9.1	14.2
16:1	9.9	9.5	5.9	10.4	8.9	10.2	9.5	10.4	12.0	9.8	11.4	11.7
16:2	1.0	1.0	Tr	1.3	1.2	2.5	1.0	1.1	Tr	1.2	1.2	Tr
17:0	2.3	2.2	Tr	2.3	1.4	1.8	2.1	1.8	1.6	2.3	1.4	3.9
18:0	4.1	3.0	12.2	4.7	3.2	9.6	4.8	3.3	10.2	4.6	3.7	11.8
18:1	6.7	6.3	4.7	8.0	8.1	7.4	8.4	8.9	10.2	8.0	8.8	9.1
18:2 Δ6.9	3.1	3.5	9.4	4.2	4.7	2.8	5.4	5.1	2.5	5.2	5.5	2.5
18:2 Δ9.12	21.2	23.3	22.0	17.5	19.3	15.8	18.6	19.6	14.7	18.3	20.4	15.8
18:3	26.8	30.3	13.7	21.6	23.1	14.0	22.2	24.2	10.1	24.0	25.4	10.3
Unsaturated	68.7	73.9	55.7	63.0	65.2	52.7	65.1	69.3	49.7	66.7	72.7	48.3

[a] Average of three experiments; Tr, traces.

Table 5. Major fatty acid composition of the individual phospholipids of the microsomal subfractions of *Tetrahymena* (% of total fatty acid)[a]

Phospho-lipid[b]	Microsomal subfractions	Fatty acids													Un-satu-rated
		12:0	14:0 iso	14:0	15:0	16:0	16:1	16:2	17:0	18:0	18:1	18:2 Δ6.9	18:2 Δ9.12	18:3	
PE	smooth I	3.3	9.8	3.3	1.3	9.5	14.2	3.1	3.9	4.3	7.5	2.5	18.5	18.8	64.6
	smooth II	3.6	8.7	2.7	1.1	7.9	14.6	3.2	2.2	2.9	8.5	4.0	19.0	21.6	70.9
	rough	3.5	8.0	2.4	1.5	6.4	12.3	1.7	2.7	2.7	8.0	4.8	21.0	25.0	72.8
GAEP	smooth I	1.4	5.8	2.3	1.2	6.6	9.1	1.3	2.3	3.0	9.0	8.4	24.0	25.6	77.3
	smooth II	1.6	4.9	3.2	1.0	8.3	8.6	Tr	2.0	2.6	9.1	10.5	24.4	23.8	76.4
	rough	2.8	7.6	2.8	1.3	6.8	8.5	1.7	Tr	2.4	9.0	8.5	23.5	25.1	76.3
PC	smooth I	4.1	7.0	3.2	5.2	9.5	11.8	1.8	3.0	3.7	7.5	3.0	19.8	20.4	64.3
	smooth II	4.7	6.2	4.8	5.9	9.3	8.7	3.6	Tr	2.3	9.8	4.2	18.6	21.9	66.8
	rough	2.1	8.6	1.0	5.1	9.8	9.7	3.2	Tr	6.0	8.5	4.5	18.1	23.4	67.4
LPC	smooth I	7.7	10.1	1.3	8.8	14.1	15.0	1.2	1.3	10.4	14.5	Tr	14.6	1.0	46.3
	smooth II	4.3	15.8	1.9	10.7	14.0	19.0	Tr	Tr	8.8	16.3	1.0	7.2	1.0	44.5
	rough	5.0	10.2	3.1	8.8	15.6	13.0	Tr	3.1	6.8	14.4	3.2	13.5	6.3	47.4
CAEP	smooth I	4.1	12.8	6.2	3.8	16.1	10.9	3.2	3.4	4.6	5.8	1.5	13.8	13.6	48.8
	smooth II	3.6	13.4	5.8	3.6	18.6	8.0	Tr	5.8	1.9	3.3	4.2	14.5	17.0	47.0
	rough	3.3	13.4	6.8	4.3	14.0	13.5	Tr	5.2	6.0	7.8	3.6	11.3	10.7	46.9
MMAEP	smooth I	3.7	13.5	5.5	5.0	15.3	9.5	2.3	3.2	4.4	8.4	4.8	11.2	13.0	49.2
	smooth II	2.9	12.3	5.0	4.6	17.2	14.0	Tr	1.4	7.1	12.3	3.0	11.0	8.5	48.8
	rough	2.7	10.6	3.0	4.8	15.6	14.0	1.2	2.0	6.6	9.4	3.2	12.0	14.8	54.6
PI	smooth I	2.0	13.3	5.1	8.6	7.5	18.3	3.1	1.7	3.4	9.4	3.8	7.4	16.4	58.4
	smooth II	1.2	13.8	9.5	8.5	12.7	13.9	Tr	Tr	3.6	9.8	3.8	8.4	15.4	51.3
	rough	1.1	14.4	6.1	7.4	14.4	13.4	Tr	1.7	3.1	8.6	2.4	8.9	18.5	51.4
CL	smooth I	3.7	7.3	3.6	2.7	10.2	10.5	2.0	3.5	5.7	9.6	3.2	18.4	19.6	63.3
	smooth II	1.3	8.6	3.0	3.1	6.8	12.5	Tr	Tr	6.4	11.3	4.3	19.7	23.0	70.8
	rough	Tr	8.5	4.8	3.6	6.6	7.5	2.0	1.1	2.5	6.5	5.4	26.0	25.5	72.9

[a] Average of two experiments. [b] For abbreviations *see* Table 1; Tr, traces.

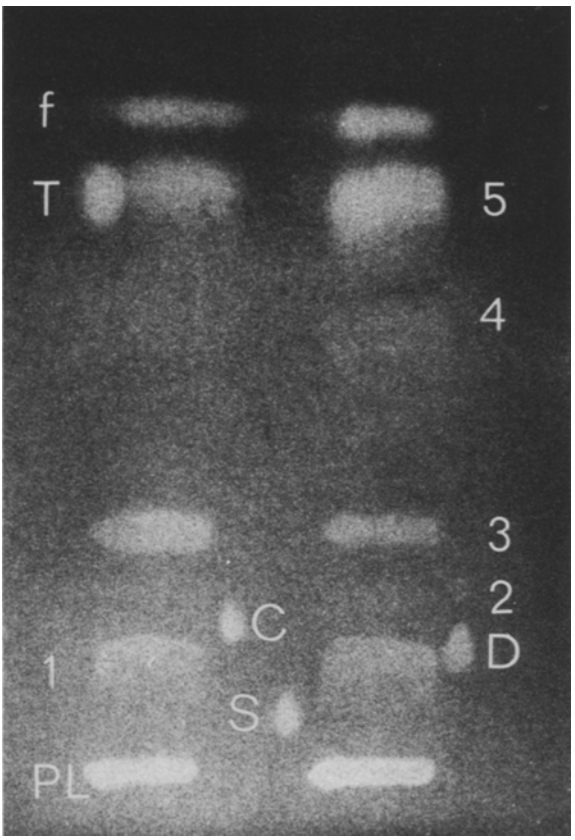

Fig. 5. Thin-layer chromatogram of the neutral lipids of whole *Tetrahymena* cells (right) and smooth I membranes (left), sprayed with tinopal and photographed at 254 nm. PL, polar lipids; 1, free fatty acids/diglyceride fraction; 2, tetrahymanol; 3, unknown NL-compound; 4, quinones; 5, triglycerides; f, front. Reference substances: S, stearic acid; D, diglyceride; C, cholesterol; T, triglyceride

Table 6. Fatty acid composition of neutral lipids of *Tetrahymena* cells and microsomal subfractions in mole % of total fatty acids[a]

Lipid	Whole cells	Microsomal subfractions		
		Smooth I	Smooth II	Rough
Diglycerides	42.1	51.2	47.2	48.4
Unknown NL-compound	18.1	28.7	33.5	32.6
Triglycerides	39.8	20.1	19.3	19.0

[a] Average of three experiments.

Table 7. Major fatty acid composition of the individual neutral lipids of the microsomal subfractions of *Tetrahymena* (% of total fatty acid)[a]

Neutral lipids	Microsomal subfractions	Fatty acids													Unsaturated
		12:0	14:0 iso	14:0	15:0	16:0	16:1	16:2	17:0	18:0	18:1	18:2 Δ6.9	18:2 Δ9.12	18:3	
Diglycerides	smooth I	3.1	11.8	4.5	1.7	18.6	10.5	2.5	Tr	6.3	7.4	2.8	15.8	15.0	54.0
	smooth II	2.0	9.8	5.6	3.2	19.5	12.0	4.4	1.0	10.3	8.5	4.4	11.3	8.0	48.6
	rough	3.3	10.8	3.1	3.4	16.0	9.0	Tr	5.5	8.3	11.9	4.2	15.4	9.0	49.5
Unknown NL-compound	smooth I	1.2	11.0	4.0	2.8	17.0	10.8	6.5	2.5	10.4	9.1	2.2	10.0	12.5	51.1
	smooth II	3.2	10.2	4.1	6.5	18.3	11.0	Tr	Tr	11.3	8.1	2.4	14.3	10.4	46.2
	rough	4.2	10.8	1.4	4.6	17.9	12.8	5.4	Tr	10.2	11.3	2.4	10.7	8.5	51.0
Triglycerides	smooth I	4.3	5.0	12.3	2.2	11.7	11.2	Tr	1.8	12.2	8.4	2.6	19.8	8.5	50.5
	smooth II	6.1	3.6	14.2	4.1	14.2	14.4	Tr	2.8	5.3	11.2	1.3	11.9	10.9	49.7
	rough	5.4	6.5	11.0	4.7	13.0	14.1	1.2	3.9	6.2	10.2	4.4	9.5	9.9	49.3

[a] Average of four experiments; Tr, traces.

the total nonpolar lipid fractions of both whole cells and microsomes (Fig. 5). This is consistent with other studies, showing a very low tetrahymanol/phospholipid ratio of 0.054 and 0.041 for whole cells and total microsomes, respectively [*see* e.g. 33].

The diglyceride fraction and the unknown NL-compound are richer in fatty acids in the microsomes than in whole cells, while the reverse is revealed with the triglycerides (Fig. 5; Table 6). Differences between whole cells and the microsomes can be also detected in the fatty acid pattern (Table 4). It is typical for the unsaturated fatty acids of the apolar lipids that they contain more octadecadienoic acids and less octadecatrienoic acid than those of the polar lipids. Moreover, the neutral lipids of the smooth I microsomes contain more unsaturated acids (52.7%) than the smooth II (49.7%) and the rough microsomes (48.3%; Table 4). Finally, the triglycerides contain about the same amount of unsaturated fatty acids (50%) in all three microsomal subfractions (Table 7). The latter reveal some minor differences concerning the amount of unsaturated acids in the diglyceride fraction and the unknown NL-compound (Table 7).

Thermotropic Response

In order to test whether the membranes in the three microsomal fractions of *Tetrahymena* retain their thermotropic character during isolation,

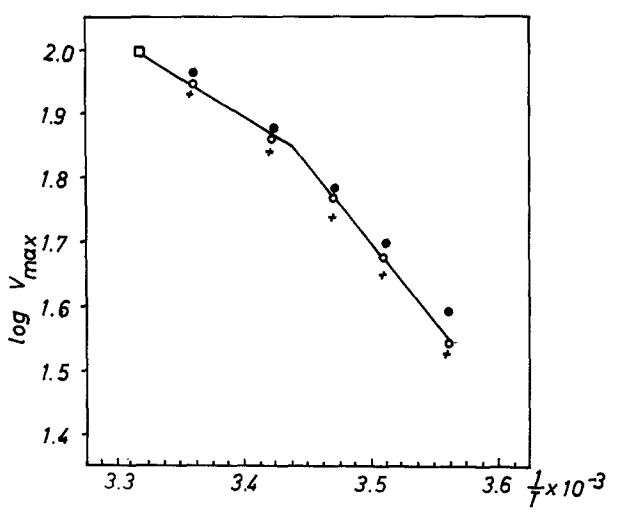

Fig. 6. Temperature dependence of the activity of the glucose-6-phosphatase associated with the smooth I (○—○), the smooth II (+—+), and the rough microsomes (●—●). The activity (Pi/min/mg protein), determined at V_{max}, is normalized to 28 °C

we measured the activity of a typical endoplasmic reticulum marker enzyme, glucose-6-phosphatase, as a function of temperature. The Arrhenius plot in Fig. 6 shows that the thermotropic response of the glucose-6-phosphatase is not linear, but rather reveals a discontinuity at $\sim 18\,^{\circ}C$ in the smooth I, smooth II, and rough membranes. Above and below this discontinuity, the activation energies lie in the range of ~ 5 kcal/mole and ~ 12 kcal/mole, respectively.

Discussion

Smooth and rough microsomes have been isolated from *Tetrahymena* by one centrifugation step on two-layered, Mg^{2+}-containing sucrose gradients; the smooth microsomes separate into a light (smooth I) and a heavy (smooth II) fraction. The ribosome-studded vesicles of the rough microsomes are obviously derived from the rough endoplasmic reticulum, whereas the exact cellular origin of the light and heavy smooth membranes is unclear. These smooth membranes reveal only a low contamination with mitochondrial membranes which is indicated by the low relative specific activity of the succinic dehydrogenase and the low content of cardiolipin. Moreover, the plasma membrane contamination appears to be low since we detect only traces of tetrahymanol, which is the typical lipid for a distinct part of the plasma membrane, i.e. the ciliary membrane [14, 33]. This view is also supported by a totally different phospholipid composition of the cilia [21, 28, 33] and the smooth microsomal membranes as well as the low relative specific activity of 5′-nucleotidase, which is widely regarded as plasma membrane marker enzyme. Since the activity of the endoplasmic reticulum marker enzyme glucose-6-phosphatase is enriched in our membrane preparations in comparison to whole cells, we assume that the greatest portion of the smooth I and smooth II membranes stems from the polymorphic smooth endoplasmic reticulum. This contributes by far the greatest portion to the total endomembranes in *Tetrahymena* and consists of membranes arranged as cisternae, vesicles, tubules, sacs and vacuoles of different size and shape possessing apparently heterogenous function [for review *see* 7, 11]. Possibly the light, smooth membranes are involved in vivo more in intracellular digestion processes since they display a higher acid phosphatase activity than the smooth II membranes.

However, rather than an exact knowledge of the intracellular origin of the microsomal membranes the fact is important that, under the isolation conditions used, at least a significant portion of the membranes have apparently retained their thermotropic character, despite the high activities of lipo- and proteolytic enzymes known to be present in *Tetrahymena* [*cf.* e.g. 21]. This is indicated by the fact that, in all three microsomal fractions, the glucose-6-phosphatase activity reveals a thermotropic discontinuity at ~18 °C. We assume that this thermal response of the microsomal membranes is comparable to the in vivo situation, since we previously observed, also at ~18 °C, thermotropic transitions directly in the cores of the *Tetrahymena* endomembranes, which were visualized by freeze-etch electron-microscopy [29, 38, 39, 42]. In general, thermotropic discontinuities of membrane-bound enzymes, including the glucose-6-phosphatase, are interpreted as being induced by a disorder⇌order phase transition of the membrane lipids [e.g. 6, 13, 17, 32]. However, the many unsaturated fatty acids in the microsomal membranes of *Tetrahymena* indicate that the thermotropic discontinuity of the *Tetrahymena* glucose-6-phosphatase cannot be simply induced by a true lipid phase transition but rather by clustering of "rigid" liquid crystalline lipids as we show elsewhere in the smooth I membranes using different physical methods such as electron-spin resonance, calorimetry, fluorescence spectroscopy and proton nuclear magnetic resonance [40].

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