Biochimica et Biophysica Acta, 775 (1984) 141–149
Elsevier

BBA 72214

HOMEOVISCOUS THEORY UNDER PRESSURE

I. THE FATTY ACID COMPOSITION OF *TETRAHYMENA PYRIFORMIS* NT-1 GROWN AT HIGH PRESSURE

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(Received March 20th, 1984)

Key words: Membrane lipid; Fatty acid composition; High pressure; Homeoviscous adaptation; (T. pyriformis)

***Tetrahymena* was grown at up to 260 atm to see if the bilayer-ordering effect of pressure increased the proportion of unsaturated fatty acids in the membrane lipids. Both whole cells and microsomes showed no such change in their fatty acid composition. The most striking effect was seen in the former which showed a pressure-dependent increase in the proportion of C16:0 in relation to C16:Δ9. Homeoviscous adaptation to pressure does not appear to occur in this cell.**

Introduction

The lipid composition of many cell membranes is regulated, apparently to maintain the fluidity of the bilayer within tolerable limits. In the case of the eukaryote *Tetrahymena pyriformis* NT-1, growth at low temperature is associated with a larger proportion of unsaturated fatty acids in the mebranes than is the case at high temperature. The change in membrane composition partially counteracts the immediate effects of temperature on bilayer fluidity as measured by spectroscopic methods. The relationship between bilayer fluidity (order parameter) and the degree of unsaturation of its constituent fatty acids is non-linear, and the adaptive significance of homeoviscous changes in membranes has yet to be demonstrated in detail.

In the case of *Tetrahymena* membranes it has been postulated that a reduction in temperature increases the order of the endoplasmic reticulum bilayer, which in turn activates the desaturase enzymes associated with it. The resultant increase in the proportion of unsaturated fatty acids reduces bilayer order, therebye reducing desaturase activity [1,2]. The hypothesis is conceived in general terms; '...it can be used to explain the fatty acid changes induced by other environmental factors, such as salinity, pressure and drugs' [2].

This paper describes experiments designed to test the hypothesis in relation to the second factor, pressure. Cells have been subjected to high pressure to ascertain whether their fatty acid composition changes in the same way as it does at low temperature. Two important complications arising from the nature of the high pressure stress have to be considered. The first is the effect of high hydrostatic pressure on the health of the cells. Spin label and fluorescent probe methods have shown that high pressure increases the order parameter in various cell membranes by an amount which may be offset by a temperature increase of 0.013–0.023 deg. C/atm [3]. However to maintain *Tetrahymena pyriformis* NT-1 viable over a period of hours, pressure has to be limited to approximately 260 atm, equivalent to a temperature decrease of between 3.4 and 6 deg. C. Thus the maximum pressure it is practical to apply is equivalent to a temperature decrease which is rather small to elicit

a convincing change in membrane composition. Additionally, pressure may cause other metabolic disturbances [4].

The second complication in this study is that of designing an experiment in which pressure is a strictly independent variable. Subjecting cells to variations in purely hydrostatic pressure requires the absence of a gas phase in order to eliminate the effects of gas partial pressures. The exclusion of a gas phase introduces severe culture limitations [5]. Helium gas may be used to apply pressure to cultures, enabling them to be aerated in the usual way in the presence of a normal partial pressure of oxygen, whilst subjected to high hydrostatic pressure. The significance of a high partial pressure of helium in conjunction with an equivalent hydrostatic pressure is probably small in the range of pressure used in this study [6].

Materials and Methods

The high temperature strain of the ciliated protozoan *Tetrahymena pyriformis* NT-1 was kindly provided by Dr. G.A. Thompson, Austen, TX. The cells were routinely grown in test tube cultures comprising Oxoid proteose peptone (2% solution) and yeast extract (0.1% solution) at 37 °C. For experimental purposes a 200 ml volume of medium in a 500 ml conical flask was inoculated with approx. 25 ml of 3-day-old test tube culture and magnetically stirred at 37 °C for approx. 30 h. Cells from the resultant mid-log phase cultures were then subjected to one of five sets of growth conditions. The cultures were (a) either kept at 37 °C or their flasks were transferred to 23 °C or 18 °C water baths, or (b) cells were dispersed in 200 ml of new, aerated medium in a 500 ml conical flask at room temperature, and then incubated at either 37 °C or 23 °C. Cultures in (a) or (b) were magnetically stirred, sterile and in contact with air in the normal way. Condition (c) comprised cells dispersed in 1.5 litre of oxygenated growth medium at room temperature to give an initial cell concentration of $(5–10) \cdot 10^3$ cells $\cdot$ ml^{-1} (see below). The culture was sealed free of bubbles, either in a plastic cylinder previously sterilised with ethanol or an autoclaved glass bottle with a narrow neck, which was subsequently sealed with a 3 cm layer of sterile liquid paraffin floating on the culture medium. These dispersed and sealed cultures were incubated at 37 °C, 23 °C or 18 °C and intermittently magneticially stirred to prevent the cells settling on the bottom. Condition (d) comprised dispersed and sealed cultures, as in (c), placed within a steel pressure vessel and pressurised with liquid paraffin. The pressure vessel was of 12 cm internal diameter $\times 40$ cm internal length and fitted with an external water jacket which enabled its temperature to be controlled at 37 ± 0.2 °C. High pressure was generated by a pneumatically powered pump connected to the vessel by a loop of flexible steel capillary tubing. The vessel was mounted on a spindle and rocked by an electrically powered crank mechanism. This caused a plastic ball (1.5 cm) to roll on the bottom of the culture vessel and prevented the cells from settling.

The growth which sealed cultures could support was estimated by comparing the increase in cell numbers in sealed and dispersed cultures at 37 °C with that of similarly dispersed cultures which were stirred in air. It was found that cell numbers increased to $35 \cdot 10^3$ cells $\cdot$ ml^{-1} before growth in the sealed cultures began to lag behind that in the aerated cultures. Accordingly the sealed cultures used for the present experiments contained a terminal cell density of no more than $15 \cdot 10^3$ cells $\cdot$ ml^{-1} acquired over 11–16 h. In such conditions oxygen or other volatile factors do not limit cell multiplication, but may, of course, influence fatty acid metabolism.

Finally, the fifth growth condition (e), comprised cells dispersed in aerated medium as in (b) and grown in an atmosphere of high pressure helium combined with a normal partial pressure of air. For these experiments, cells were transferred from mid log phase cultures to give $20 \cdot 10^3$ cells $\cdot$ ml^{-1} in 200 ml of medium in a 500 ml conical flask plugged with cotton wool. The cultures were stirred by a magnetic follower at 37 °C and 23 °C $\pm$ 0.2 °C. For incubation at high pressure (37 °C) a flask was placed in an 11 cm bore vertically mounted pressure vessel fitted with an internal electric motor to provide magnetic stirring, a thermistor and an exhaust valve. Pressure was applied in stepwise fashion from a gas pump fed from a standard cylinder of helium, (99.995%) added to

the air-filled pressure vessel at an average rate of 250 atm · h^{-1}. The temperature of the compressed gas transiently exceeded 40 °C during the early stages of compression. Decompression was carried out over 1 h to minimise foaming. The decompression profile comprised an initial rapid decrease to 50 atm, irrespective of incubation pressure, followed by steps at 10 min intervals to 25, 12, 6 and 3 atm and then finally to normal atmospheric pressure. The cells were fixed with formalin at the start of decompression by means of a catheter tube inserted into the top of the culture flask, but clear of the medium. The catheter tube passed inside a high pressure pipe and connected through an isolating valve to a small satellite pressure vessel. When the satellite vessel was filled with formalin and the isolating valve opened, the initial decompression of the main vessel sucked formalin into the culture to give a final concentration of 2%. Control cultures comprised, as before, dispersed cultures grown in stirred flasks open to the air at 37 °C and 23 °C.

In summary, the fatty acids from cells incubated in five different types of culture are compared;

(a) Non-dispersed cultures, in air, normal pressure, 18 °C, 23 °C and 37 °C.

(b) Dispersed cultures, in air, normal pressure, 23 °C and 37 °C.

(c) Dispersed and sealed cultures, normal pressure, 18 °C, 23 °C and 37 °C.

(d) Dispersed and sealed cultures, 260 atm hydrostatic pressure, 37 °C.

(e) Dispersed cultures, 260, 170 and 73 atm helium pressure, 37 °C.

Typically two growth experiments were carried out in parallel, combining control and experimental conditions in different ways, e.g. conditions (a) and (b), (c) and (d) and (b) and (e), etc.

After fixation in 2% formalin [7] the cells were counted with a Coulter Counter and examined by light microscopy before harvesting. Phospholipids were extracted by established methods and the fatty acid methyl esters analysed by gas chromatography (Carlo-Erba Fractovap 4160) using hydrogen as carrier gas and a capillary coated with SP1000. Each fatty acid was identified by mixing with a known standard to produce a single peak except in the case of 20:1Δ15? which was provisionally identifed on the basis of retention time (see Tables I and II). Chromatograms were routinely analysed by a Hewlett Packard reporting integrator.

Additionally microsomes were prepared from cells grown in cultures (b) at 23 °C and 37 °C, and in culture (e) at 262 atm [1]. Steady-state fluorescence polarisation of diphenylhexatriene-labelled microsomes was determined, using a Perkin-Elmer 3000 spectrometer, and the fatty acid composition of the microsomes was also determined.

Results

The composition of the main fatty acids in the cells is given in Tables I and II and may be conveniently summarised by three ratios; (i) the combined weight per cent of the saturated fatty acids : unsaturated fatty acids (saturation ratio), (ii) the weight per cent of C16:0/C16:1Δ9 (C16 ratio), and similarly (iii) the ratio of C18:0/C18:1Δ9 (C18 ratio). These ratios provide an index of the steady-state balance between the fatty acids at the time of sampling.

Cells grown in control, non-dispersed cultures (condition (a)) at 37 °C, 23 °C and 18 °C have the fatty acid composition shown in Table I (lines A, B and C). Their fatty acid composition demonstrates the importance of the physiological state of the cells, which is determined by aeration (stirring), temperature, and the cycle of growth in batch culture. In the non-dispersed condition the cells are entering late log phase, their multiplication rate is low and their fatty acid composition, listed in Table I, shows no significant temperature dependence.

Dispersal of the cells and incubation in cultures in contact with air (condition b) elicits a faster rate of multiplication and the cells' fatty acid composition shows greater temperature dependence. The saturation ratio in cells grown at 37 °C is significantly higher than that in cells grown at 23 °C, and similarly the C16 ratio at 37 °C is higher than at 23 °C. However the C18 ratio shows the reverse (Table I, lines D, E, and ratios 1, 2 and 3). Growth in sealed cultures (condition (c)) reduces the overall rate of multiplication but both the saturation ratio and C16 ratio differ between cells grown at 37 °C and 23 °C. (Table I, lines F and G, ratios 4

TABLE I

THE MAIN FATTY ACIDS IN *TETRAHYMENA PYRIFORMIS* NT-1 GROWN AT HIGH HYDROSTATIC PRESSURE AND UNDER CONTROL CONDITIONS

Figures are mean weight % (±S.D.). Lines labeled A, B, etc. and ratios [1], [2], etc. are referred to in the text.

Growth conditions (see Methods), duration, increase in cell number, n = number of experiments	14:0	15:0	16:0	16:1 Δ9	16:2 Δ10, 13	18:0	18:1 Δ9	18:1 Δ11	18:1 Δ13	18:2 Δ9, 12	18:3 Δ6, 9, 12	20:1 Δ15?	20:2 Δ11, 14	Mean ratios (±S.D.) sat./unsat	16:0/16:1Δ9	18:0/18:1Δ9
(a) Non-dispersed, open, atmospheric pressure																
A 37–36 °C, 19.3 (±2.6) h, Increase 1.8±0.47-fold to (173±87)·10^3 cells·ml^{-1}, n = 7	6.75 (4.8)	0.81 (0.03)	21.3 (3.0)	2.25 (1.22)	2.03 (0.27)	5.84 (2.4)	15.7 (3.5)	1.22 (0.10)	0.76 (0.12)	16.9 (3.1)	13.5 (4.9)	4.5 (3.5)	3.41 (1.9)	0.61 (0.31)	11.9 (6.5)	0.39 (0.17)
B 23 °C, 17.5 (±0.8) h, Increase 1.18±0.9-fold to (153±18.5)·10^3 cells·ml^{-1}, n = 3	3.64 (3.5)	0.30 (0.30)	13.0 (1.7)	2.94 (0.93)	2.15 (0.54)	2.62 (0.53)	7.80 (1.8)	1.74 (0.64)	1.34 (0.16)	23.2 (4.2)	25.4 (5.1)	5.84 (3.7)	2.60 (0.90)	0.26 (0.07	4.6 (0.81)	0.34 (0.04)
C 18 °C, 19.2 (±3.8) h, Increase 1.0±0.3-fold to (99±91)·10^3 cells·ml^{-1}, n = 2	0	0	9.73 (1.9)	6.30 (3.2)	1.70 (0.57)	1.87 (0.70)	8.10 (0.40)	0	0.82 (0.38)	20.2 (1.2)	25.7 (10)	0.96 (0.45)	4.37 (1.2)	0.25 (0.15)	1.88 (0.91)	0.23 (0.07)
(b) Dispersed, open, atmospheric pressure																
D 37 °C, 12.6 (±1.4) h, Increase 5.8±3-fold to (75±66)·10^3 cells·ml^{-1}, n = 4	5.40 (3.5)	0.89 (0.60)	14.9 (2.7)	5.85 (1.0)	2.36 (1.6)	2.90 (2.6)	13.1 (3.8)	0	0.77 (2.3)	14.3 (7.3)	15.3 (1.5)	6.59 (8.3)	3.40 (0.50)	0.40 [1] (0.06)	2.6 [2] (0.55)	0.26 [3] (0.08)

E 23 °C, 12.1 (±0.8) h, Increase 2.47-fold to $(27 \pm 17) \cdot 10^3$ cells·ml^{-1}, $n = 4$	3.87 (2.8)	0.56 (0.13)	11.7 (2.3)	9.14 (2.9)	1.90 (0.90)	2.10 (1.8)	5.70 (3.2)	0	1.67 (0.54)	15.3 (3.2)	22.7 (2.9)	6.40 (3.50)	2.50 (0.80)	0.27 [1] (0.03)	1.40 [2] (0.46)	0.34 [3] (0.09)
(c) Dispersed, sealed, approx. atmospheric, pressure																
F 37 °C, 17 atm, 11.4 (±7) h Increase 2.8 ± 1.3-fold to $(14.3 \pm 9.7) \cdot 10^3$ cells·ml^{-1}, $n = 3$	6.32 (5.0)	1.3 (0.10)	17.2 (3.1)	5.4 (1.4)	1.5 (0.10)	2.90 (0.40)	5.60 (1.6)	0	0	18.0 (5.8)	22.0 (3.9)	5.90 (6.3)	2.90 (1.0)	0.44 [4] (0.06)	3.40 [5] (1.0)	0.53 [7] (0.12)
G 23 °C atmospheric pressure 12.8 (±1.8) h, Increase 1.6 ± 0.5-fold to $(13.2 \pm 3.3) \cdot 10^3$ cells·ml^{-1}, $n = 4$	2.57 (1.2)	0.75 (0.17)	13.2 (1.7)	8.40 (2.2)	1.36 (0.19)	2.37 (0.34)	5.9 (1.5)	0	1.41 (0.30)	19.3 (5.6)	24.4 (6.0)	4.1 (2.0)	2.57 (0.40)	0.26 [4] (0.02)	1.65 [5.6] (0.46)	0.49 (0.18)
H 18 °C atmospheric pressure 16.6 (±1.1) h, Increase 1.08 ± 0.07-fold to $(15.1 \pm 0.23) \cdot 10^3$ cells·ml^{-1}, $n = 3$, unstirred	0.41 (0.30)	0.75 (0.60)	12.6 (3.2)	5.80 (2.0)	1.31 (0.14)	2.77 (0.90)	18.2 (10)	0	0.63 (0.01)	15.5 (8.7)	14.8 (5.4)	0.74 (0.38)	3.80 (1.3)	0.40 (0.14)	2.59 [8] (1.4)	0.16 [7] (0.04)
(d) Dispersed, sealed, 262 ± 3.8 atm																
I 37 °C, 13.6 (±1.6) h, Increase 1.12 ± 0.4-fold to $(10.2 \pm 2.6) \cdot 10^3$ cells·ml^{-1}, $n = 5$	1.18 (1.4)	0	15.1 (5.2)	1.98 (0.50)	2.60 (0.50)	7.50 (4.1)	11.4 (8.6)	0	0.60 (0.60)	11.1 (6.7)	15.8 (6.3)	7.60 (4.7)	6.50 (2.9)	0.44 (0.23)	8.1 [6.8] (2.8)	1.01 (0.78)

TABLE II

THE MAIN FATTY ACIDS IN *TETRAHYMENA PYRIFORMIS* NT-1 GROWN AT HIGH PARTIAL PRESSURES OF HELIUM AND UNDER CONTROL CONDITIONS

Figures are mean weight % (±S.D.). Lines labelled A, B, etc. and ratios [1], [2], etc. are referred to in the text.

Growth conditions (see methods), duration, increase in cell number, n = number of experiments	14:0	15:0	16:0	16:1 Δ9	16:2 Δ10, 13	18:0	18:1 Δ9	18:1 Δ11	18:1 Δ13	18:2 Δ9, 12	18:3 Δ6, 9, 12	20:1 Δ15?	20:2 Δ11, 14	Mean ratios (±S.D.) sat./unsat.	16:0/16:1Δ9	18:0/18:1Δ9
(b) Dispersed, open, atmospheric pressure																
A 37 °C, 16.9 (±2.7) h, Increase 10.09±3.8-fold to (143±76)·10^3 cells·ml^{-1}, n = 9	5.17 (2.4)	0.78 (0.57)	13.5 (2.7)	4.47 (1.2)	3.23 (1.2)	3.58 (1.8)	16.5 (6.1)	1.03 (0.44)	0.76 (0.35)	11.56 (3.8)	15.7 (2.8)	5.41 (3.7)	3.03 (1.7)	0.37 [1,4,5] (0.08)	3.36 [2,6,7] (1.6)	0.24 [3,11,12] (0.13)
B 37 °C, 18 h, Increase 2.45-fold to 40·10^3 cells·ml^{-1}, n = 2, unstirred	0.68 –	0.45 –	14.3 –	4.45 –	2.13 –	2.36 –	16.78 –	1.48 –	0 –	16.3 –	26.1 –	3.77 –	2.69 –	0.24 –	3.40 –	0.14 –
C 23 °C, 18.02 (±1.81) h, Increase 2.54±0.74-fold to (66.5±46.14)·10^3 cells·ml^{-1}, n = 10	2.84 (1.9)	0.30 (0.29)	12.0 (2.2)	8.32 (2.53)	2.24 (0.80)	1.90 (0.92)	5.15 (1.1)	1.25 (0.50)	1.70 (0.80)	15.4 (0.69)	24.2 (3.1)	6.76 (6.9)	3.75 (2.1)	0.23 [1] (0.06)	1.57 [2] (0.62)	0.42 [3,9,10] (0.12)
(e) Dispersed, open, high pressure helium																
D 37 °C, 73±1.8 atm p_{He}, 17.9 (±1.5) h, Increase 4.9±1.1-fold to 103·10^3 cells·ml^{-1}, n = 3	2.62 (1.1)	0.53 (0.75)	18.9 (1.9)	3.08 (1.1)	3.0 (0.39)	8.57 (2.3)	9.37 (1.9)	0	0	15.1 (1.7)	21.9 (1.6)	3.10 (1.1)	4.03 (2.4)	0.51 [5] (0.03)	6.94 [6,8] (3.4)	0.97 [9,11] (0.46)
E 37 °C, 168±7 atm p_{He}, 17.5 (±1.08) h, Increase 1.26±0.22-fold to (42±18)·10^3 cells·ml^{-1}, n = 3	3.10 (4.7)	1.47 (1.5)	13.8 (1.3)	1.87 (0.64)	2.19 (0.79)	3.60 (2.4)	4.36 (1.0)	0	0	13.4 (3.6)	27.1 (5.4)	11.1 (8.3)	6.29 (2.6)	0.31 (0.03)	8.03 (2.9)	0.76 (0.42)
F 37 °C, 221 atm p_{He}, 17 h, Increase 0.25-fold to 12·10^3 cells·ml^{-1}, n = 2, unstirred	3.1 –	0 –	16.7 –	1.84 –	1.74 –	3.04 –	5.28 –	1.02 –	0 –	8.20 –	16.7 –	19.7 –	9.79 –	0.35 –	9.23 –	0.57 –
G 37 °C 262±5.5 atm p_{He}, 13.6 (±2.5) h, Increase 0.75±0.09-fold to (33±11)·10^3 cells·ml^{-1}, n = 4	2.16 (1.5)	0.57 (0.67)	21.6 (5.1)	1.74 (0.33)	3.32 (1.6)	7.40 (1.3)	5.11 (1.5)	0	0	5.70 (1.3)	12.6 (5.0)	12.2 (9.5)	8.13 (1.4)	0.65 [4] (0.22)	12.4 [7,8] (1.8)	1.50 [10,12] (0.34)

and 5). At 18°C the cells have intermediate saturation and C16 ratios but a C18 ratio which is significantly lower than that in 37°C cells (Table I, lines F and H, ratio 7).

Hydrostatic pressure

Growth in sealed cultures pressurised hydrostatically to a mean pressure of 262 atm at 37°C (condition (d)) produces cells with saturation and C18 ratios which are not significantly different from those in cells grown sealed at 37°C, normal pressure (Table I, lines F and I). The C16 ratio in the pressurised cells is higher than in the sealed control cells, but only with a statistical significance level which lies just short of $P = 0.02$ (Table I, lines F and I). When the data for the fatty acids in the pressurised cells are compared with those of cells grown at 23°C in the sealed condition, differences are apparent. Thus the saturation ratios differ slightly and the C16 ratios significantly (Table I, lines G and I, ratio 6). Cells at 18°C have a significantly lower C16 ratio than those at 262 atm, with a similar multiplication rate (Table I, lines H and I, ratio 8). The C18 ratio at 18°C or 23°C is not significantly different from that at 262 atm (Table I, lines G, H and I).

In summary, whole cells grown in sealed cultures at 37°C and 23°C manifest significantly different saturation and C16 ratios. Cells growing in sealed cultures at 37°C and 262 atm differ little in their fatty acid composition from cells at 37°C, normal pressure, but they differ rather more from cells grown sealed at 23°C, and 18°C, normal pressure. In particular the change in the C16 ratio at high pressure is qualitatively inconsistent with the hypothesis outlined in the introduction.

Helium pressure

The results of experiments using high helium pressures are given in Table II. A comparison of the results of the control experiments for this series with those in Table I provides a reassuring measure of reproducibility (Table II, lines A and C compared with Table I, lines D and E). As in the first series of experiments the fatty acid composition of the cells shows temperature dependence when cell numbers increase (Table II, lines A and C, ratios 1 and 2). Note the C18 ratio in 37°C cells is lower than in 23°C cells, as in Table I (Table II, ratio 3). In the absence of stirring at 37°C the growth rate is reduced and so is the saturation ratio (Table II, lines A and B).

Growth in high pressure helium at 37°C alters the saturation ratio. 73 and 262 atm increase the ratio whereas the intermediate pressure of 168 atm does not (Table II, lines A, D, F and G, ratios 4 and 5). This is clearly unlike the effect of reducing temperature. The C16 ratio is increased by high pressures of helium (Table II, lines A, B, D, E, F and G, ratios 6, 7 and 8). Fig. 1 demonstrates the linear increase in C16 ratio with helium pressure and the comparison with the hydrostatic pressure effect in sealed cultures.

The C18 ratio of cells grown at 73 atm and 262 atm is higher than that of cells grown at 37°C, normal pressure (Table II, lines A, D and G, ratios 11 and 12). It is also higher than that of cells grown at 23°C, normal pressure (Table II, lines C, D and G, ratios 9 and 10).

In summary, in whole cells high pressure helium increases both saturation and C16 ratios, whereas reducing the temperature at normal pressure decreases them. In its effect on the C16 ratio helium pressure resembles hydrostatic pressure (Fig. 1). High helium pressure increases the C18 ratio, with

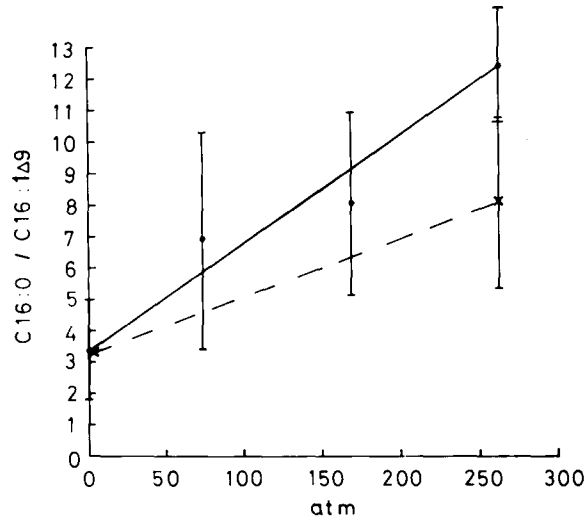

Fig. 1. The ratio (weight per cent) of the fatty acids C16:0/C16:1Δ9 in cells grown at 37°C in (a), (●) high helium pressure and (b), (×) high hydrostatic pressure. The lines are drawn by inspection.

TABLE III

FATTY ACID RATIOS IN MICROSOMAL MEMBRANES FROM CELLS GROWN UNDER DIFFERENT CONDITIONS

Figures are means ± S.D.

Growth conditions	$\frac{\text{sat.}}{\text{unsat.}}$	$\frac{C16:0}{C16:1\Delta9}$	$\frac{C18:0}{C18:1\Delta9}$
Conditions (b) (see text) normal atmospheric pressure			
37 °C	0.36 ± 0.11	9.17 ± 3.67	0.54 ± 0.23
23 °C	0.29 ± 0.15	3.02 ± 2.56	0.67 ± 0.48
Conditions (e) (see text) 250 atm helium pressure			
37 °C	0.36 ± 0.07	10.5 ± 2.42	0.89 ± 0.26

73 atm or more at 37 °C exerting a much bigger effect than a reduction in temperature to 23 °C at normal pressure.

Microsomes

The fatty acid composition of the microsomal membranes obtained from cells grown in high pressures of helium is summarised in Table III.

The C16 ratio is significantly lower in the microsomes obtained from cells growing at 23 °C as compared with those from 37 °C atmospheric pressure. Cells grown at 37 °C and 250 atm helium pressure show no such effect.

The values for the steady-state fluorescence polarisation of diphenylhexatriene-labelled membranes at 7.5 °C were as follows. Microsomes from cells grown at 37 °C gave a mean polarisation of 0.353 ± 0.018 (S.D.) which may be compared to 0.426 ± 0.013 (S.D.) for hand-shake liposomes of pure dipalmitoylphosphatidylcholine. Cells grown at 23 °C gave microsomal polarisation values of 0.347 ± 0.015 (S.D.). Cells grown at 37 °C at 250 atm helium pressure yielded microsomes whose mean polarisation value was 0.337 ± 0.013 (S.D.). In short, no significant differences were detected by the fluorescence polarisation method.

Discussion

Cells at 260 atm helium pressure (condition (e)) showed a decline in number whilst those in 260 atm hydrostatic pressure (condition (d)) did not, so clearly the two conditions for growth differ. However, in view of the biochemical inertness of helium, and its marginal effects at this pressure [6], the view is taken here that helium probably exerted little effect in these experiments. The elevated C16 ratios seen in both conditions (Fig. 1) is most simply accounted for as a purely hydrostatic pressure effect, which is unlike the effects of reducing temperature to 23 °C at atmospheric pressure. Changes in the C18 saturation ratio are not well defined. The differences between the fatty acids in the two conditions, helium and hydrostatic pressure, are most likely caused by culture conditions, perhaps aeration.

Tetrahymena's homeoviscous regulatory mechanism therefore does not respond to 260 atm helium or purely hydrostatic pressure as it does to a reduction of 14 °C. Significantly, the C16 and saturation ratios in microsomes from cells grown at 260 atm helium pressure do not differ from those of the controls. This does not necessarily mean the 'cold-activation' hypothesis of homeoviscous regulation in *Tetrahymena* mentioned in the Introduction is disproved, since pressure could be affecting other metabolic pathways which obscure the results. It does, however, suggest that the homeoviscous regulation of membrane lipids in *Tetrahymena* subjected to experimental high pressure does not occur.

The fluidity and fatty acid composition of membranes from deep sea fish tissues from 4000 m depth (i.e. 400 atm) have been shown to be more fluid at normal pressure and more unsaturated than controls, suggesting that homeoviscous adaptation to high pressure occurs in Nature [8].

Acknowledgements

I thank Miss Alison Stewart for skilled technical assistance, Dr. J. Sargent for advice on lipid analyses, and Dr. D. Burke for the use of a fluorescence spectrometer.

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