CHANGES IN LIPID COMPOSITION OF EHRLICH ASCITES TUMOR CELLS INDUCED BY CULTIVATION IN MEDIA WITH INCREASED SODIUM CHLORIDE CONTENT

Heinz EGGE *, Dietrich SCHACHTSCHABEL** and Fritz ZILLIKEN*

Institut für Physiologische Chemie, Rheinische Friedrich-Wilhelms-Universität, Bonn
and
Institut für Physiologische Chemie II, Philipps-Universität, Marburg, Germany

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1. Introduction

Cell cultures of hyperdiploid Ehrlich ascites tumor cells have been adapted, by stepwise increase of the sodium chloride content of Eagle's medium, to growth and multiplication in hypertonic media with a salt concentration of 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 M, respectively [1, 2]. Sublines of these cultures have been serially subcultivated in the respective hypertonic media. The increased sodium chloride milieu caused alterations in cellular morphology, growth rate, enzyme pattern and macromolecular synthesis [1–4].

Considerations about possible effects of the high salt environment on metabolism and structure of cellular membranes prompted us to analyse the lipid composition of different sublines of these highly salt tolerant cells.

2. Material and methods

The origin and maintenance of the predominantly hyperdiploid (42 – 45 chromosomes per metaphase) Ehrlich ascites tumor cells as well as the procedure for adapting these parent cells (ED 0.15 - cells) to growth and multiplication in hypertonic media has been described [1, 2, 5]. The present cells were cultured in media containing a salt concentration (moles/l) of 0.15 ("control" medium), 0.25, 0.30, 0.35, 0.40 and 0.45, respectively. "Control" cells were grown in Eagle's basal medium (Grand Island Biol. Co.) supplemented with 10% fetal calf serum (Grand Island Biol. Co.), 100 I.U./ml penicillin and 100 μg/ml streptomycin. The media with increased sodium chloride content were prepared by addition of appropriate amounts of a 2.15 M NaCl stock solution to the culture media [1]. The stock solution was prepared by dissolving NaCl (Suprapur; Fa. Merck) in the culture medium. Cultures were prepared by seeding approx. 10^5 cells in 10 ml volumes of culture medium in 4-ounce pharmacy bottles (Fa. Brockway Glass Company) containing an atmosphere of 7% CO_2, 20% O_2 and 73% N_2. The spent medium was replaced with 15 ml of fresh, prewarmed (37°) medium every 24 to 48 hr and the incubation was maintained until total cell protein amounted to approx. 2 mg per culture. The last medium renewal was performed always 1 hr prior to lipid extraction. After the cell sheet had been washed 3 times with 10 ml of Earle's salt solution, containing appropriate amounts of NaCl, total lipids were extracted according to Folch [6] and determined gravimetrically [7]. The distribution of neutral lipids and phospholipids was carried out according to a modified method of Van Gent [7, 8]. Protein was determined according to Lowry [9] using bovine albumin (Fa. Sigma) as standard. Analysis of the fatty acid composition of total lipids was carried out by gas liquid chromatography after transesterifica-

* Institut für Physiologische Chemie, 53 Bonn, Nussallee 11, Germany.
** Institut für Physiologische Chemie II, 355 Marburg, Auf den Lahnbergen, Germany.

Table 1
Lipid composition of Ehrlich ascites tumor cells serially cultivated in isotonic (salt concentration: 0.15 M) or hypertonic media with different salt concentrations (0.25, 0.30, 0.35, 0.40, or 0.45 M).

Salt concentration of the medium (M)	Lipid composition of cells (in % of total lipid): μg lipid / mg protein	CHE	TG	CH	FFA	C.L.	P.E.	P.C.	SP+I.P.	Tot. P.L.
0.15	181 (± 6)	18.0 (± 3)	7.8 (± 2.5)	10.8 (± 0.7)	3.5 (± 1.4)	3.5 (±0.9)	14.9 (± 1.2)	26.9 (± 2.2)	15.6 (± 0.5)	59.8 (± 3.1)
0.25	190 (± 19)	14.5 (± 1.5)	6.8 (± 0.6)	11.5 (± 1.1)	4.1 (± 0.7)	6.4 (± 1.4)	17.7 (± 1.5)	25.1 (± 1.7)	17.3 (± 2.5)	63.0 (± 3.2)
0.30	226 (± 23)	5.6 (± 0.8)	13.4 (± 1.9)	10.6 (± 0.8)	4.0 (± 1.2)	6.3 (± 1.9)	17.6 (± 3.0)	30.7 (± 2.2)	16.1 (± 0.7)	66.5 (± 1.3)
0.35	245 (± 25)	3.2 (± 1.6)	10.6 (± 5)	9.7 (± 1.1)	3.2 (± 0.6)	4.8 (± 1.3)	17.3 (± 3.5)	26.1 (± 3.0)	17.8 (± 2.0)	65.2 (± 6.0)
0.40	235 (± 26)	6.8 (± 0.9)	18.4 (± 5)	8.8 (± 1.4)	3.8 (± 0.5)	4.2 (± 1.0)	12.8 (± 2.5)	28.5 (± 2.5)	16.0 (± 1.2)	63.0 (± 2.0)
0.45	239 (± 42)	9.2 (± 1.3)	13.1 (± 2)	10.0 (± 1.1)	5.0 (± 0.7)	5.4 (± 0.6)	16.4 (± 2.5)	26.8 (± 1.5)	10.5 (± 1.5)	64.0 (± 4.0)

Abbreviations: CHE = cholesterolesters; TG = triglyceride; CH = cholesterol; FFA = free fatty acids; C.L. = cardiolipin; P.E. = phosphatidylethanolamine; P.C. = phosphatidylcholine; S.P. = sphingomyelin; I.P. = inositolphosphatide; P.L. = phospholipids. The mean values are given for 4 cultures each. The lipid distribution of every culture flask was determined in duplicate. The figures in brackets give the mean deviation.

tion with methanolic HCl using a 50 m × 0.25 mm capillary column coated with ethylenglycolsuccinate-polyester (Perkin Elmer). A Carlo Erba gas chromatograph and a Kent Chromalog integrator were used. Samples of 10 ng were injected with nanojectors (Applied Science Kit Co. Pennsylvania, USA) directly on the capillary column without splitting.

3. Results

Determination of the total cellular lipid content relative to the protein content (lipid/protein ratio) of Ehrlich ascites tumor cells, derived from cell cultures with differential concentrations of sodium chloride in the culture media, revealed an increase of the lipid/protein ratios in response to the elevation of the salt concentration in the culture media (table 1). The distribution of the individual classes of cellular lipids in correlation with the salt concentration of the culture medium is summarized in table 1. The relative amounts of cholesterol and free fatty acids (FFA) are remarkably constant and not significantly influenced by changes of environmental conditions. Only a slight but not significant rise in cellular FFA levels was observed at higher salt concentration of the culture media (table 1).

The increased salt content in the culture medium considerably influences the amount of cellular cholesterolesters and triglycerides. Thus there was a relative decrease of cholesterolesters and an increase of triglycerides in response to the elevation of the NaCl content in the culture media (table 1).

Besides the changes in individual lipid classes, alterations in fatty acid distribution were observed (table 2). Due to the low quantities of fatty acids obtained, only the major components could be evaluated with sufficient accuracy. The amounts are presented as relative values, stearic acid methylester corresponding

Table 2
Major fatty acid composition of total lipids (after transesterification) of Ehrlich ascites tumor cells and fetal calf serum.

Fatty acid	Major fatty acid composition of lipids derived from: Cells serially cultivated in media with the following salt concentration (M): 0.15	0.25	0.35	0.40	0.45	Fetal calf serum
$C_{16:0}$	122.1 (± 0.41)	90.0 (± 2.9)	66.5 (± 2.1)	68.9 (± 3.5)	62.4 (± 0.9)	286.9 (± 17.7)
$C_{16:1}$	41.2 (± 1.2)	27.1 (± 3.1)	14.8 (± 1.4)	15.4 (± 0.5)	13.1 (± 2.7)	114.0 (± 4.6)
$C_{18:0}$	100.0	100.0	100.0	100.0	100.0	100.0
$C_{18:1}$	329.0 (± 23.2)	155.7 (± 11.0)	150.2 (± 5.9)	153.5 (± 5.7)	142.5 (± 5.9)	356.3 (± 21.0)
$C_{18:2}$	4.19 (± 1.5)	4.38 (± 1.5)	6.19 (± 3.8)	12.7 (± 3.6)	11.9 (± 4.7)	48.3 (± 20.9)

The cells are derived from cell cultures which were adapted to proliferation in media with different salt concentrations. The amounts are expressed in relative values, stearic acid corresponding to 100.0. The values represent the means (± the mean square deviation) of the determination of 5–7 preparations of each cell sample.

to 100.0. The elevation of the salt concentration of the culture media has resulted in relative decreases of the cellular content of palmitic, palmitoleic and oleic acid (table 2). Contrary to the relative decreases in the content of these fatty acids, an increase in the cellular content of linoleic acid in response to the elevated salt environment was observed (table 2).

4. Discussion

If the cultivation of genetically "identical" cells in media altered with respect to only a single salt constituent such as NaCl has resulted in permanent changes in the lipid composition of these cells, such cellular alterations may be directly related to the altered ionic composition of the media.

The observed increase of total cellular lipids relative to total protein at elevated salt concentrations of the culture media is well correlated with the strongly increased activity of enzyme systems supplying reduced NADP [3, 4]. Although the role of glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) in the regulation of lipid synthesis is still contradictory [10], its strongly enhanced activity [3, 4] in these "high-NaCl"-tolerant cells may also be interpreted as a sign of increased lipid synthesis.

The increase in the cellular content of triglycerides and the decrease in the content of cholesterolesters in these "high-NaCl"-tolerant cells (table 1) is not understood on a molecular level, at present. It may be assumed that these changes reflect a cellular response to the strained osmotic conditions. Since the lipid composition of these cells might be influenced, at least in part, by the lipid composition of the culture medium containing fetal calf serum, a lipid analysis of fetal calf serum was of particular interest. However, comparison of the lipid composition of cultured Ehrlich ascites tumor cells and fetal calf serum revealed only a slight similarity (tables 1 and 2; fig. 1). Therefore, it is unlikely that a major exchange between extra- and intracellular lipids occurs in these cells.

In spite of increased activities of enzyme systems which supply reduced NADP [3, 4], the desaturation of fatty acids and/or the incorporation of monoenoic acids into cellular lipids seems to be strongly reduced at higher salt concentrations (table 2). There is evidence that in biomembranes the hydrocarbon chains of lipids

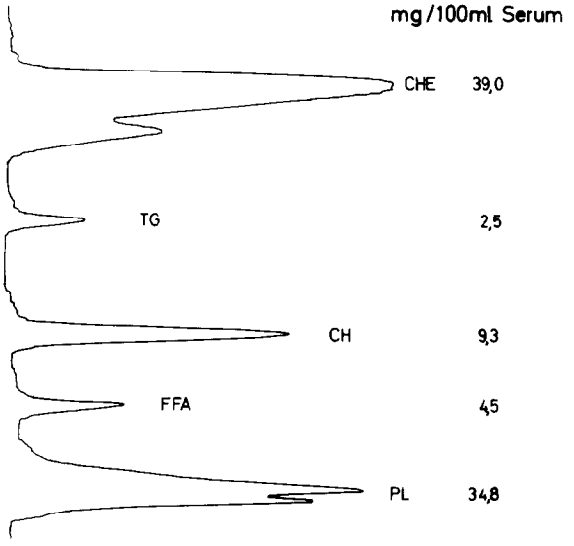

Fig.1. Densitometer curve of the lipids of fetal calf serum after thin-layer chromatography on micro chromatoplates and quantitative distribution (mg/100 ml serum) of the lipid classes.

are to a certain extent present in a liquid crystalline state, which is necessary for a proper functioning of transport processes [11–14]. Phase transition temperatures are elevated by increased saturation of fatty acid chains [11–14]. It can be assumed that the transport processes of cells grown at higher salt concentrations might be severely influenced, due to the enhanced amount of cellular saturated fatty acids. The rise in the cellular linoleic acid content following the elevation of NaCl concentration in the culture media (tables 1 and 2) suggests that the cellular uptake and utilization of this essential fatty acid is also influenced by the environmental salt milieu.

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