J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 361–368

Studies on the Effects of Betamethasone, *L*-Carnitine, and Betamethasone-*L*-Carnitine Combinations on the Dipalmitoyl Phosphatidylcholine Content and Phosphatidylcholine Species Composition in Foetal Rat Lungs

By *A. Lohninger*

Department of Medical Chemistry, University of Vienna (Austria)

H. P. Krieglsteiner

Department of Obstetrics and Gynaecology, Malteser-Krankenhaus Bonn-Hardtberg (F.R.G.)

H. Salzer, E. Vytiska-Binstorfer

I. Department of Obstetrics and Gynaecology, University of Vienna (Austria)

W. Riedl and *W. Erhardt*

Department of Experimental Surgery, Technical University of Munich (F.R.G.)

Dedicated to Prof. Dr. Erich Kaiser for his 60th birthday

(Received July 31/October 14, 1985//January 20, 1986)

Summary: Administration of *L*-carnitine or betamethasone to pregnant rats failed to increase either the total phospholipid or dipalmitoylphosphatidylcholine (DPPC) contents in foetal rat lungs on the 20th day of gestation, compared to controls. The combined administration of betamethasone (0.3 mg/kg) and *L*-carnitine (80 mg/kg) resulted in a pronounced increase of dipalmitoylphosphatidylcholine (7.8 ± 2.5 mg/g dry weight) compared with the control group (5.4 ± 1.8 mg/g dry weight), and compared with the groups receiving betamethasone (5.9 ± 1.9 mg/g dry weight) or *L*-carnitine (5.6 ± 1.5 mg/g dry weight) alone. The proportion of dipalmitoylphosphatidylcholine in the phosphatidylcholine species increased from 20.9 ± 2.1% in the foetal lungs of the control group to 22.6 ± 5.0% in the *L*-carnitine group, to 24.3 ± 3.3% ($p < 0.01$) in the betamethasone-*L*-carnitine (20 mg/kg) group, to 25.2 ± 3.5% ($p < 0.01$) in the betamethasone group, to 27.1 ± 2.6% ($p < 0.01$) in the betamethasone-*L*-carnitine (40 mg/kg) group, and to 28.4 ± 3.7% ($p < 0.01$) in the betamethasone-*L*-carnitine (80 mg/kg) group, while the palmitic acid portion in the phosphatidylcholine fatty acids was nearly unchanged. A pronounced increase of palmitoyl-myristoyl phosphatidylcholine (PC-30), the second disaturated phosphatidylcholine species present in lungs in significant amounts beside dipalmitoylphosphatidylcholine, was noted only in betamethasone treated animals. Furthermore, after betamethasone and betamethasone-*L*-carnitine treatment, a significant diminution ($p < 0.01$) of the proportion of palmitoyl-palmitoleyl phosphatidylcholine (16 : 0/16 : 1-PC) in the phosphatidylcholine species was demonstrated. After *L*-carnitine and betamethasone-*L*-carnitine treatment a significant increase ($p < 0.01$) of the proportion of palmitoleyl-palmitoyl phosphatidylcholine (16 : 1/16 : 0-PC) in the phosphatidylcholine species was found. Administration of *L*-carnitine to pregnant rats (either alone or in combination with betamethasone) resulted in a significant elevation ($p < 0.01$) of the carnitine levels in the foetal lungs to approximately twice those of the controls.

The results suggest that a betamethasone-*L*-carnitine combination has both additive effects and effects specific for the combination, neither of which are found when carnitine or betamethasone is administered alone.

Studien über die Wirkung von Betamethason, L-Carnitin und Betamethason-L-Carnitin-Kombinationen auf den Dipalmitoylphosphatidylcholingehalt und die Zusammensetzung der Phosphatidylcholin-Spezies von fetalen Rattenlungen

Zusammenfassung: Die Verabreichung von *L*-Carnitin oder von Betamethason an gravide Wistarratten führte zu keinem Anstieg des Gesamtphospholipid- und Dipalmitoylphosphatidylcholingehalts in den fetalen Rattenlungen im Vergleich zur Kontrollgruppe. Andererseits wurde nach Applikation einer Betamethason (0,3 mg/kg)-*L*-Carnitin (80 mg/kg) Kombination ein deutlicher Anstieg des Dipalmitoylphosphatidylcholingehalts (7,8 ± 2,5 mg/g Trockengewicht) im Vergleich zur Betamethason- (5,9 ± 1,9 mg/g Trockengewicht), *L*-Carnitin- (5,6 ± 1,5 mg/g Trockengewicht) und der Kontrollgruppe (5,4 ± 1,8 mg/g Trockengewicht) gefunden. Der Prozentanteil des Dipalmitoylphosphatidylcholins an den Phosphatidylcholinspezies erhöhte sich von 20,9 ± 2,1% (Kontrollgruppe) auf 22,6 ± 5,0% nach Verabreichung von *L*-Carnitin, auf 25,2 ± 3,5% ($p < 0,01$) nach Betamethasonbehandlung und nach Gaben von Betamethason-*L*-Carnitin Kombinationen bei 20 mg/kg *L*-Carnitin auf 24,3 ± 3,3% ($p < 0,01$), bei 40 mg/kg *L*-Carnitin auf 27,1 ± 2,6% ($p < 0,01$) und auf 28,4 ± 3,7% ($p < 0,001$) mit 80 mg *L*-Carnitin. Trotz des signifikanten Anstiegs des Dipalmitoylphosphatidylcholins zeigte der prozentuale Anteil der Palmitinsäure an den im Phosphatidylcholin veresterten Fettsäuren nur geringe Veränderungen. Nur in der Betamethasongruppe findet sich ein deutlicher Anstieg von Palmitoyl-myristoyl-phosphatidylcholin (PC 30), neben Dipalmitoylphosphatidylcholin die zweite mengenmäßig wichtige gesättigte Phosphatidylcholinspezies. Außerdem kam es nach Betamethasonbehandlung sowie nach Applikation von Betamethason-*L*-Carnitin Kombinationen zu einer signifikanten ($p < 0,01$) Verminderung des Anteils von Palmitoyl-palmitoleyl-phosphatidylcholin (16 : 0/16 : 1-PC) am Phosphatidylcholin. Nach *L*-Carnitin-Behandlung und nach maternaler Applikation einer Betamethason-*L*-Carnitin-Kombination wurde ein signifikanter Anstieg ($p < 0,01$) des prozentualen Anteils von Palmitoleyl-palmitoylphosphatidylcholin (16 : 1/16 : 0-PC) am Phosphatidylcholin gefunden. Applikation von *L*-Carnitin sowohl allein, als auch in Kombination mit Betamethason an gravide Ratten führte zu einem signifikanten Anstieg ($p < 0,01$) des Gesamt-Carnitingehalts in den fetalen Lungen. Diese Gehalte waren annähernd doppelt so hoch wie jene der unbehandelten oder mit Betamethason behandelten Tiere.

Die Ergebnisse der vorliegenden Studie weisen auf zwei unterschiedliche Wirkungen einer Betamethason-*L*-Carnitin-Kombinationsbehandlung hin: erstens solche, die einer additiven oder subtraktiven Wirkung der beiden Einzelkomponenten entsprechen, und zweitens Effekte, die offensichtlich spezifisch für eine Betamethason-*L*-Carnitin-Kombination sind.

Introduction

Surfactant deficiency is one of the most important factors underlying the development of the respiratory distress syndrome (RDS) in the immature lung of the newborn. Dipalmitoylphosphatidylcholine (DPPC) constitutes the major fraction of the surfactant complex and is mainly responsible for the surface active properties (1–3).

Acceleration of foetal lung development with exogenous glucocorticoids has been demonstrated in a large number of animal models, with various species, using physiological, morphological, and biochemical parameters. Such experimental findings have supported the clinical use of glucocorticoids in cases of imminent premature delivery or when progressive intrauterine damage makes premature delivery necessary. In the recent past corticosteroid prophylaxis of foetal respiratory distress syndrome has been increasingly discussed with regard to efficacy and possible side effects.

We have previously shown (4) that antepartum administration of *L*-carnitine or betamethasone enhances the dipalmitoylphosphatidylcholine content in the lungs of foetuses delivered on the 21st gestational day. The results suggested that *L*-carnitine and betamethasone affect the dipalmitoylphosphatidylcholine level in foetal lung via two dissimilar mechanisms.

In the present study we attempted to examine the effects of maternal treatment with betamethasone-*L*-carnitine combinations in comparison to the administration of *L*-carnitine and betamethasone alone. To evaluate the effects of the different treatments, we determined both the dipalmitoylphosphatidylcholine content and the proportion of dipalmitoylphosphatidylcholine in the total phosphatidylcholine of foetal rat lungs.

Materials and Methods

Seventy-five female Wistar rats of stock Ch bb-Thom with an average weight of 300 g and an expected gestation period of 23 days were randomized and divided into six groups. The gestational age was known within 24 hours. The rats were treated intraperitoneally from the 16th to the 19th day of gestation by the following schedule:

Group	Betamethasone (mg/kg)	*L*-Carnitine (mg/kg)	Solute
Controls	—	—	1 ml/day physiological saline
Betamethasone	0.3	—	1 ml/day distilled water
L-Carnitine	—	20	1 ml/day distilled water
Bethamethasone + *L*-Carnitine	0.3	20	1 ml/day distilled water
	0.3	40	1 ml/day distilled water
	0.3	80	1 ml/day distilled water

Higher carnitine doses (60, 80, 100 mg/kg) showed effects which were not significantly different from those with 20 mg/kg body weight.

Preparation of lung tissue

Immediately after delivery the foetal trachea was clamped before spontaneous inspiration could occur. The foetuses were thoracotomized by means of parasternal incision. The foetal lungs were then grouped according to litter in order to preclude station-related differences. The foetal lungs were homogenized, extracted, and washed using the method of *Folch* et al. (5).

Phospholipid analysis

Total phospholipids were assayed by a modified version of *Bartlett*'s method (6). The main phospholipid classes were separated as bands by thin-layer chromatography using the solvent system chloroform/methanol/10 g/l potassium chloride solution (volumes, 43 + 47 + 4) (4). 1,2 Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and the glycero-3-phosphocholine species composition were assayed as the corresponding diacylglycerol trimethylsilylether derivatives by gas-liquid chromatography with glass capillary columns (7, 8). Phosphatidylcholine fatty acids were determined as methyl ester derivatives by gas-liquid chromatography with glass capillary columns.

Carnitine assay

The tissue was flash-frozen immediately after removal. The perchloric acid extracts were used for assaying free and short-chain acylcarnitine. The carnitine esters were saponified and assayed as free carnitine by radioenzymatic means (9), with two modifications: HEPES instead of TRIS buffer (10), and N-ethylmaleimide instead of tetrathionate (11).

Chemicals

"*L*-Carnitin-Leopold" was obtained from Leopold Ltd. (Graz, Austria). Chloroform, methanol, pyridine, hexamethyldisilazane, thin-layer chromatography plates (silica gel 60), and HEPES were obtained from E. Merck (Darmstadt, F. R. G.). *Bacillus cereus*-derived phospholipase C and carnitine acetyltransferase were obtained from Boehringer-Mannheim (F. R. G.). Labeled acetylcoenzyme A was obtained from New England Nuclear (Boston, Mass.). N-ethylmaleimide and dimyristoyl-sn-glycero-3-phosphocholine were supplied by Sigma Chemical Company (St. Louis, MO.).

Statistical analysis

Statistical comparisons between the control group and the treated groups were made using analysis of variance followed by *Dunnet*'s t-test for multiple comparison.

Results

Total phospholipid content

Only small differences in the total phospholipid content in foetal rat lungs were found in the different treatment groups (data not shown).

Dipalmitoylphosphatidylcholine

Both betamethasone and *L*-carnitine treatments induced only a moderate increase in the dipalmitoylphosphatidylcholine content compared with the control group. However, the combined administration of betamethasone and *L*-carnitine resulted in a marked increase in the dipalmitoylphosphatidylcholine content (tab. 1). The proportion of dipalmitoylphosphatidylcholine in the phosphatidylcholine species exhibited a significant rise ($p < 0.01$) in the betamethasone group, and the betamethasone-*L*-carnitine combination groups. Only a moderate response to *L*-carnitine administration was noted (tab. 1).

Phosphatidylcholines containing palmitic and palmitoleic acid (PC-32 monoenic species)

We have previously shown that the PC-32 monoenic species can be characterized as 16 : 0/16 : 1-PC and as 16 : 1/16 : 0-PC (4). A relatively high portion of 16 : 1/16 : 0-PC in the phosphatidylcholine species is probably characteristic for foetal lungs (4) and amniotic fluid (12). The portions of 16 : 0/16 : 1-PC and of 16 : 1/16 : 0-PC in the phosphatidylcholine species of the foetal lungs are shown in table 1.

16 : 0/16 : 1-PC is significantly lower ($p < 0.01$) in the betamethasone-treated group than in the control group. A moderate diminution was found after *L*-carnitine administration. Interestingly, in the beta-

Tab. 1. PC-32 species in foetal rat lungs*)

Treatment	n	Dipalmitoylphosphatidylcholine (16 : 0/16 : 0-PC) (mg/g dry weight)	Percentage of PC-32 species in total phosphatidylcholines 16:0/16:0-PC (%)	16:0/16:1-PC (%)	16:1/16:0-PC (%)
NaCl (Controls)	16	5.4 ± 1.8	20.9 ± 2.1	4.2 ± 1.1	8.4 ± 1.6
Betamethasone (0.3 mg/kg)	14	5.9 ± 1.9	25.2 ± 3.5[1])	2.9 ± 0.8[2])	8.5 ± 1.4
Carnitine (20 mg/kg)	6	5.6 ± 1.5	22.6 ± 5.0	3.7 ± 1.1	10.9 ± 4.3
Betamethasone + Carnitine (20 mg/kg)	12	6.6 ± 1.6	24.3 ± 3.3[1])	3.2 ± 0.8[2])	9.2 ± 1.8
Betamethasone + Carnitine (40 mg/kg)	10	6.5 ± 1.7	27.1 ± 2.6[1])	2.4 ± 0.4[2])	9.9 ± 0.9[1])
Betamethasone + Carnitine (80 mg/kg)	12	7.8 ± 2.5	28.4 ± 3.7[1])	2.8 ± 0.7[2])	10.1 ± 1.5[1])

*) Values are mean ± S. D., with n = number of experiments (PC-32, total carbon atoms in acyl residues is 32).
[1,2]) Significantly higher (lower) ($p < 0.01$) compared with the control values.

methasone-*L*-carnitine groups (20 mg/kg and 40 mg/kg) there is a further decrease, whereas after administration of the betamethasone-*L*-carnitine (80 mg/kg) combination, the same level as in the betamethasone group was found.

No increase was found in the proportion of 16 : 1/16 : 0-PC following betamethasone treatment, as compared with the control group, whereas a marked increase was found after *L*-carnitine administration. The betamethasone-*L*-carnitine combination groups showed significantly ($p < 0.01$) enhanced levels.

In quantitative terms (mg/g dry weight), the same effects described above were found for both 16 : 0/16 : 1-PC and 16 : 1/16 : 0-PC (data not shown).

Composition of the phosphatidylcholine species (tab. 2)

Palmitoyl-myristoyl phosphatidylcholine (PC-30): most commonly used methods for surfactant determination are based on the determination of disaturated phosphatidylcholine, which consists of dipalmitoylphosphatidylcholine and PC-30. It was therefore of interest to examine whether the different treatments would affect dipalmitoylphosphatidylcholine exclusively, or both disaturated species. As shown in table 2, betamethasone treatment resulted in an increase of the PC-30 portion in the phosphatidylcholine species compared with the controls, whereas no response to *L*-carnitine was noted. It is of interest that the betamethasone-*L*-carnitine (40 mg/kg) combination resulted in a moderate enhancement, whereas in the betamethasone-*L*-carnitine (20 mg/kg and 80 mg/kg) combination groups no response was noted.

Composition of the phosphatidylcholine fatty acids

Table 3 shows the relative composition of the esterified fatty acids in foetal lung phosphatidylcholine. We have previously shown that each fatty acid is present in more than one phosphatidylcholine species

Tab. 2. Relative phosphatidylcholine species composition in foetal rat lungs*).

Treatment	n	PC-30 (%)	PC-32 (%)	PC-34 (%)	PC-36 (%)	PC-38 (%)
NaCl (Controls)	16	7.1 ± 2.5	33.5 ± 3.3	33.7 ± 2.8	19.2 ± 2.6	6.3 ± 1.5
Betamethasone (0.3 mg/kg)	14	8.9 ± 2.0	36.5 ± 4.7	29.3 ± 2.3	17.5 ± 2.7	8.0 ± 3.4
Carnitine (20 mg/kg)	6	7.2 ± 1.6	37.1 ± 8.4[1])	30.8 ± 2.9[3])	17.4 ± 1.3	7.2 ± 4.8
Betamethasone + Carnitine (20 mg/kg)	12	6.9 ± 2.6	36.9 ± 4.2[1])	30.4 ± 3.6	17.7 ± 2.6	8.9 ± 1.7
Betamethasone + Carnitine (40 mg/kg)	10	7.9 ± 1.8	39.4 ± 2.9[2])	29.0 ± 2.4[4])	16.7 ± 3.0	6.9 ± 1.9
Betamethasone + Carnitine (80 mg/kg)	12	7.1 ± 1.3	40.6 ± 4.7[2])	28.0 ± 3.3[4])	16.5 ± 2.7	7.6 ± 2.3

*) Values are mean ± S. D., with n = number of experiments (PC-30 etc., total carbon atoms in acyl residues is 30 etc.).
[1]) Significantly higher ($p < 0.05$) compared with the control values.
[2]) Significantly higher ($p < 0.01$) compared with the control values.
[3]) Significantly lower ($p < 0.05$) compared with the control values.
[4]) Significantly lower ($p < 0.01$) compared with the control values.

Tab. 3. Relative composition of esterified phosphaditylcholine fatty acids in foetal rat lungs*).

Treatment	n	14 : 0 (%)	16 : 0 (%)	16 : 1 (%)	18 : 0 (%)	18 : 1 (%)	18 : 2 (%)	20 : 4 (%)
NaCl (Controls)	15	3.7 ± 0.8	39.2 ± 1.7	9.8 ± 1.0	10.6 ± 1.5	21.7 ± 1.5	4.8 ± 0.6	7.3 ± 1.4
Betamethasone (0.3 mg/kg)	13	3.7 ± 0.7	40.8 ± 2.0	9.5 ± 1.1	11.0 ± 1.7	18.5 ± 0.9[1])	6.0 ± 0.8[3])	7.6 ± 1.3
Carnitine (20 mg/kg)	6	4.0 ± 0.8	41.3 ± 4.0	10.3 ± 1.2	9.7 ± 1.6	19.6 ± 1.5[2])	4.6 ± 0.6	7.3 ± 1.3
Betamethasone + Carnitine (20 mg/kg)	16	3.5 ± 0.5	42.2 ± 2.3	9.8 ± 0.9	8.5 ± 1.3	19.5 ± 3.3[2])	5.7 ± 0.5	7.2 ± 1.3
Betamethasone + Carnitine (40 mg/kg)	8	3.7 ± 0.6	41.6 ± 1.9	9.7 ± 0.7	9.5 ± 1.4	18.0 ± 1.0[1])	5.4 ± 0.6	7.8 ± 0.7
Betamethasone + Carnitine (80 mg/kg)	13	4.1 ± 1.0	41.5 ± 2.7	9.3 ± 1.2	9.8 ± 1.8	19.1 ± 2.3[1])	5.8 ± 0.7[3])	7.4 ± 1.9

*) Values are mean ± S. D., with n = number of experiments. 14 : 0, myristic acid; 16 : 0, palmitic acid; 16 : 1, palmitoleic acid; 18 : 0, stearic acid; 18 : 1, oleic acid; 18 : 2, linoleic acid; 20 : 4, arachidonic acid.
[1]) Significantly lower ($p < 0.01$),
[2]) significantly lower ($p < 0.05$),
[3]) significantly higher ($p < 0.01$) compared with the control group.

(7, 8). For instance, palmitic acid (16 : 0) occurs in PC-30, in the two PC-32 monoenic species (16 : 0/16 : 1-PC and 16 : 1/16 : 0-PC), in dipalmitoylphosphatidylcholine (16 : 0/16 : 0-PC), and represents as 16 : 0/18 : 1-PC the dominant fraction of PC-34 (8). Thus, it is not surprising that the alterations in the phosphatidylcholine fatty acid pattern are less pronounced compared with the marked changes of the phosphatidylcholine species composition (tab. 2). In spite of the fact that many studies on the stimulation of surfactant synthesis are based on the portion of palmitic acid in total fatty acids of lung phosphatidylcholine, it is of interest to note that in the present study, no accordance between the proportion of palmitic acid and the dipalmitoylphosphatidylcholine content was found (tab. 1, 3).

Carnitine

Table 4 shows the content of total carnitine in the foetal lungs. Betamethasone failed to influence the carnitine content. Maternal *L*-carnitine administration (20 mg/kg) resulted in a significant increase ($p < 0.01$). The same carnitine level was attained following treatment with 20 mg/kg or with 40 mg/kg betamethasone-*L*-carnitine. Administration of a betamethasone-*L*-carnitine (80 mg/kg) combination resulted in a further, but not dose-related increase. The proportion of short-chain acyl-carnitine in the total carnitine was relatively constant in all groups (data not shown).

Tab. 4. Total *L*-carnitine content in foetal rat lungs*).

Treatment	n	Total *L*-carnitine (nmol/g frozen weight)
NaCl (Controls)	16	116 ± 27
Betamethasone (0.3 mg/kg)	14	119 ± 33
Carnitine (20 mg/kg)	6	222 ± 47[1])
Betamethasone + Carnitine (20 mg/kg)	12	215 ± 24[1])
Betamethasone + Carnitine (40 mg/kg)	10	218 ± 56[1])
Betamethasone + Carnitine (80 mg/kg)	12	262 ± 91[1])

*) Values are mean ± S. D., with n = number of experiments.
[1]) Significantly higher ($p < 0.01$) compared to the control group and the betamethasone-treated group.

Foetal weight and foetal lung weight (tab. 5)

As shown in numerous animal experiments maternal treatment with corticosteroids resulted in a decrease of foetal weight and in a marked reduction of the foetal lung weight (13 – 16). Also, in the present study the lowest foetal body weights and foetal lung weights were found in the betamethasone-treated group (tab. 5). Administration of *L*-carnitine resulted in an enhancement of both foetal weight and foetal lung weight ($p < 0.01$) compared with the control group and the other treatment groups. Interestingly, treatment with the betamethasone-*L*-carnitine (80 mg/kg) combination resulted in a reduction of the foetal lung weight comparable to that of the betamethasone group.

Tab. 5. Body weight and lung weight of the foetal rats*).

Treatment	n	Body weight (g)	Lung weight (mg)
NaCl (Controls)	17	3.0 ± 0.6	58.2 ± 12.9
Betamethasone (0.3 mg/kg)	14	2.6 ± 0.5	48.6 ± 11.2
Carnitine (20 mg/kg)	6	3.3 ± 0.8[2])	78.0 ± 26.7[1])
Betamethasone + Carnitine (20 mg/kg)	14	2.6 ± 0.9	54.8 ± 15.3
Betamethasone + Carnitine (40 mg/kg)	10	2.8 ± 0.4	58.8 ± 13.8
Betamethasone + Carnitine (80 mg/kg)	15	2.8 ± 0.7	50.8 ± 13.8

*) Values are mean ± S.D., with n = number of experiments.
[1]) Significantly higher ($p < 0.01$) compared with all other experimental groups.
[2]) Significantly higher ($p < 0.01$) compared with the values of the betamethasone, and the betamethasone + carnitine groups.

Discussion

Weinhold et al. (17) have shown that 50 to 60% of foetal rats delivered two days prematurely survive when placed in an incubator, while foetuses delivered three days before term did not survive. In the present study the foetal rats were delivered three days before term, in contrast to previous studies in which delivery was performed two days prematurely (4).

Betamethasone

It has been reported that relatively low doses of betamethasone (0.25 mg/kg) given to the pregnant rabbit increase the rate of choline incorporation into phosphatidylcholine by foetal lung. However, larger doses maternally administered cause foetal death, apparently due to premature aging of the placenta (18). In the present study no increase in total phospholipid- and dipalmitoylphosphatidylcholine-contents with respect to dry weight were found after administration of 0.3 mg/kg betamethasone to the mother animals. This is in accordance with studies on rhesus monkeys (19, 20). *Funkhouser* et al. (21) showed that dexamethasone exposure does not increase the total amount of surfactant per lung.

It is of interest to note that betamethasone treatment also influences palmitoyl-myristoyl phosphatidylcholine (PC-30) both in quantitative and relative terms (tab. 2). The role of PC-30 is unclear. *Goerke* et al. (22) reported that PC-30 did not meet with all of the criteria of surfactant material.

As reported previously (4) the two monoenic PC-32 species (16 : 0/16 : 1-PC and 16 : 1/16 : 0-PC) are of special interest, because the types of phosphatidylcholine that they contain are different in foetal lungs and the lungs of adult animals (tab. 1).

These results were substantiated by the study of *Longmuir* et al. (23). An unusually high portion of palmitoleic acid (16 : 1), which was more than one-fourth of the total fatty acids of the lamellar body phosphatidylcholine from foetal rabbit lungs, was found.

Carnitine

In contrast to glucocorticoids, no toxic side effects are produced by *L*-carnitine (*L*-3-hydroxy-4-N-trimethylaminobutyrate) (24, 25). In addition to the well established role of carnitine in the oxidation of fatty acids in mitochondria, several additional functions of carnitine in cell metabolism have been reported (summarized recently, (4)).

In the rat foetus plasma (26) and tissue (27, 28) levels of both free and short-chain acyl-carnitine are low and increase up to parturition. As in the foetal rat, the rate of carnitine synthesis is low in the human foetus (29). Furthermore, maternal plasma carnitine concentrations are strongly reduced during pregnancy (29–31).

The rate of fatty acid oxidation is low during the intrauterine phase (32). Shortly after parturition there is a striking increase in energy production by fatty acid oxidation due to the interrupted maternal glucose supply. At this time tissue carnitine levels must be sufficiently high for free fatty acid mobilization from endogenous stores (33, 34) as well as for fatty acid oxidation in various tissues, accompanied in the liver by ketone body production (35). It is of interest to note that the rate of fatty acid synthesis from acetoacetate is two or three times greater than from glucose in developing rat lung (36). Therefore, immediately after parturition, a sufficient amount of

ketone bodies is necessary for the synthesis of surfactant precursors to meet the need for surfactant postnatally.

Administration of *L*-carnitine to pregnant rats elevated the carnitine in foetal lungs to a level approximately twice that of the controls (tab. 4) and roughly equivalent to that of untreated animals at term.

Carnitine-betamethasone combination

The combined administration of betamethasone and *L*-carnitine resulted in two different effects: additive or subtractive effects, and effects apparently specific for the combination.

Using a standard betamethasone dose of 0.3 mg/kg the best results were obtained in combination with 80 mg/kg *L*-carnitine. The enhancement of the dipalmitoylphosphatidylcholine content of foetal lungs is obviously specific for the combination, since this effect was not found after betamethasone or *L*-carnitine treatment alone (tab. 1). However, there was rather an additive effect of the betamethasone-*L*-carnitine combination on PC-30 and 16 : 1/16 : 0-PC.

Little is known about interactions of carnitine and glucocorticoids. In studies on carnitine transport across the plasma membrane an increased uptake in the presence of prednisolone was noted (37, 38). In human systemic carnitine deficiency, corticosteroid treatment is known to be beneficial (39). It is also possible that the effect of the betamethasone-*L*-carnitine combination is mediated by other mechanisms. *Chaudhary* et al. (40) found an increased cyclic AMP (cAMP) level in foetal rat lungs after dexamethasone treatment. An important role of cAMP in the regulation of surfactant production by foetal lungs has been postulated (41). Glucocorticoids promote the conversion of foetal thyroxine (T_4) to triiodothyronine (T_3) rather than reverse T_3, and this could account for the increase in T_3 levels (42). Accelerated lung maturation after treatment either with T_4 or T_3 has been reported. Since T_4 enhances the activity of enzymes involved in carnitine metabolism it was also of interest to investigate the effect of an *L*-carnitine-thyroxine combination. The results of these experiments will be published elsewhere.

Acknowledgement

The authors wish to express their appreciation to *Barbara Lohninger* for technical assistance and for typing the paper.

This work was supported by grants from the Allianz Insurance Company, F. R. G., and by the Bürgermeisterfonds der Bundeshauptstadt Wien.

References

1. Funkhouser, J. D. & Hughes, E. R. (1978) Biochim. Biophys. Acta *530*, 9–16.
2. Akino, T., Kawamoto, T. & Ohno, K. (1978) Tohoku J. Exp. Med. *124*, 307–322.
3. Hildebran, J. N., Goerke, J. & Clements, J. A. (1979) J. Appl. Physiol. *47*, 604–611.
4. Lohninger, A., Krieglsteiner, P., Nikiforov, A., Erhardt, W., Specker, M., Martin, G. & Kaiser, E. (1984) Pediatr. Res. *18*, 1246–1252.
5. Folch, J., Lees, M. & Stanley, G. H. (1957) J. Biol. Chem. *226*, 497–503.
6. Bartlett, G. R. (1959) J. Biol. Chem. *234*, 466–468.
7. Lohninger, A. & Nikiforov, A. (1980) J. Chromatogr. *192*, 185–192.
8. Nikiforov, A., Lohninger, A., Specker, M. & Linhart, L. (1982) Eur. J. Mass Spectrom. Biochem. Med. Environ. Res. *2*, 105–112.
9. Cederblad, G. & Lindstedt, S. (1972) Clin. Chim. Acta *37*, 235–241.
10. Christiansen, R. Z. & Bremer, J. (1976) Biochim. Biophys. Acta *448*, 562–568.
11. Cederblad, G., Finnstroem, O. & Martensson, J. (1982) Biochem. Med. *27*, 260–265.
12. Lohninger, A., Salzer, H., Simbruner, G., Husslein, P. & Martin, G. (1983) Clin. Chem. *29*, 650–655.
13. Mostello, D. J., Hamosh, M. & Hamosh, P. (1981) Biol. Neonate *40*, 121–128.
14. Ballard, P. L. (1979) Monographs Endocrinol. *12*, 493–515.
15. Tsai, M. Y., Josephson, M. W. & Brown, D. M. (1981) Biochim. Biophys. Acta *664*, 174–181.
16. Mitzner, W., Johnson, J. W. C., Beck, J., London, W. & Sly, D. (1982) Am. Rev. Respir. Dis. *125*, 233–238.
17. Weinhold, P. A., Quade, M. M., Broyowski, T. B. & Feldman, D. A. (1980) Biochim. Biophys. Acta *617*, 76–84.
18. Brehier, A., Benson, B. J., Williams, M. C., Mason, R. J. & Ballard, P. L. (1977) Biochem. Biophys. Res. Commun. *77*, 883–890.
19. Beck, J. C., Johnson, J. W. C., Mitzner, W., Lee, P. A., London, W. T. & Sly, D. L. (1981) Am. J. Obstet. Gynecol. *139*, 465–470.
20. Johnson, J. W. C., Mitzner, W., London, W. T., Palmer, A. E., Scott, R. & Kearney, K. (1978) Am. J. Obstet. Gynecol. *130*, 905–910.
21. Funkhouser, J. D. & Hughes, E. R. (1980) Biochim. Biophys. Acta *619*, 506–514.
22. Goerke, J. (1974) Biochem. Biophys. Acta *344*, 241–261.
23. Longmuir, K. J., Resele-Tiden, C. & Sykes, A. (1985) Biochim. Biophys. Acta *833*, 135–143.
24. Tao, R. C. & Yoshimura, N. N. (1980) J. Parenter. Enter. Nutr. *4*, 469–479.
25. Wolff, J., Miller, D. M. & Strack, E. (1971) Acta Biol. Med. Germ. *26*, 1237–1242.
26. Seccombe, D., Hahn, P. & Novak, M. (1978) Biochim. Biophys. Acta *528*, 483–489.
27. Borum, P. R. (1978) Biochem. J. *176*, 677–681.
28. Borum, P. R. (1981) Nutr. Review *39*, 385–390.

29. Bargen-Lockner, C., Hahn, P. & Wittman, B. (1981) Am. J. Obstet. Gynecol. *140*, 412–414.
30. Hahn, P. (1982) Nutr. Res. *2*, 201–206.
31. Shenai, J. P., Borum, P. R., Mohan, P. & Donlevy, S. C. (1983) Pediatr. Res. *17*, 579–582.
32. Novak, M., Monkus, E. F., Chung, D. & Buch, M. (1981) Pediatrics *67*, 95–100.
33. Novak, M., Penn-Walker, D., Hahn, P. & Monkus, E. F. (1975) Biol. Neonate *25*, 85–94.
34. Novak, M., Penn-Walker, D. & Monkus, E. F. (1975) Biol. Neonate *25*, 95–107.
35. Hahn, P. (1982) Ann. Rev. Nutr. *2*, 91–111.
36. Sheehan, P. M. & Yeh, Y.-Y. (1984) Lipids *2*, 103–108.
37. Bohmer, T. & Molstad, P. (1980) in: Carnitine Biosynthesis, Metabolism, and Functions. (Frenkel, R. E. & Mc Garry, J. D., eds.) Academic Press, N. Y., pp. 73–89.
38. Molstad, P. & Bohmer, T. (1979) Biochim. Biophys. Acta *585*, 94–99.
39. Mc Garry, D. J. & Foster, D. W. (1980) N. Engl. J. Med. *303*, 1413–1415.
40. Chaudhary, K. C. & Nijar, M. S. (1984) Biol. Neonate *45*, 188–196.
41. Aeberhard, E. E., Barrett, C. T., Kaplan, S. A. & Scott, M. L. (1985) Biochim. Biophys. Acta *833*, 473–483.
42. Liggins, G. C. (1976) Am. J. Obstet. Gynecol. *126*, 931–935.

A. Lohninger
Med.-chem. Institut
der Universität Wien
Währinger Straße 10
A-1090 Wien