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Comparison of the Effects of Betamethasone and L-Carnitine on Dipalmitoylphosphatidylcholine Content and Phosphatidylcholine Species Composition in Fetal Rat Lungs

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ABSTRACT. Antepartum administration of L-carnitine to pregnant rats results in an increase of both total phospholipid (80 ± 11 mg/g dry weight (dw); mean ± SD) and dipalmitoylphosphatidylcholine (DPPC) content (7.7 ± 2.1 mg/g dw) in fetal lungs as compared to controls (72 ± 10 and 7.0 ± 2.5 mg/g dw, respectively). On the other hand, no effect of L-carnitine could be demonstrated on the DPPC portion in the total phosphatidylcholine (PC) or on the portion of palmitic acid in the PC fatty acids.

Betamethasone elevated the DPPC content in fetal lungs (8.1 ± 2.0 *versus* 7.0 ± 2.5 mg/g dw in the controls), while total phospholipid content remained unaffected (71 ± 19 *versus* 72 ± 10 mg/g dw). The portion of DPPC in the PC species increased significantly ($p < 0.01$) from 27.6 ± 4.5 in the fetal lungs of the control group to 34.2 ± 3.3 in the lungs of the betamethasone-treated group, while the palmitic acid portion in the PC fatty acids was nearly unchanged (45.9 ± 3.2 *versus* 43.9 ± 2.6 in the controls). Further, after betamethasone treatment, a significant diminution ($p < 0.01$) of the monoenic PC 32 species (palmitoyl-palmitoleyl PC and palmitoleyl-palmitoyl-PC) and the PC 34 species (consisting primarily of palmitoyl-oleoyl-PC) could be demonstrated both in absolute and relative terms. This is in agreement with a significant reduction of the portions of palmitoleic ($p < 0.01$) and oleic ($p < 0.05$) acids in the total PC fatty acids. The findings suggest two different mechanisms of DPPC elevation in fetal rat lungs for L-carnitine and betamethasone. (*Pediatr Res* 18:1246–1252, 1984)

Abbreviations

DPPC, dipalmitoylphosphatidylcholine
PC, phosphatidylcholine
PC-30, total carbon atoms in acyl radicals is 30
PC-32, total carbon atoms in acyl radicals is 32
PC-34, total carbon atoms in acyl radicals is 34
GLC[2], gas-liquid chromatography with glass capillary columns
MS, mass spectrometry
***t*-BDMS, *t*-butyldimethylsilyl**
TMS, trimethylsilyl
16:0/16:1-PC, palmitoyl-palmitoleyl-PC
16:1/16:0-PC, palmitoleyl-palmitoyl-PC
dw, dry weight
HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

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Acceleration of fetal pulmonary maturation processes in cases of imminent premature delivery or when progressive intrauterine damage makes premature delivery necessary is a not yet solved problem. Liggins *et al.* (26, 27) were the first to report on the acceleratory effect of maternally administered glucocorticoids on fetal lung maturation. These findings were subsequently substantiated by numerous studies on various species. A similar effect on fetal lung maturation has also been demonstrated for several other hormones: adrenocorticotropic hormone (46), thyrotropin releasing hormone (42), thyroxine (44, 52), 17β-estradiol (39), and prolactin (20). Animal experiments and clinical observations suggest that β-adrenergic agonists, such as isoxsuprine (24) and hexoprenaline (28), and cholinergic agonists, such as oxotremorine (1), also promote fetal lung maturation.

The use of carnitine appears to be of special interest, since it produces no known toxic side effects (47, 51). Both carnitine (L-3-hydroxy-4-*N*-trimethylaminobutyrate) and its acyl esters are found in living cells. Its role in the oxidation of fatty acids by mitochondria is well established (14). However, the extramitochondrial occurrence of acetylcarnitine and octanoylcarnitine transferase activities in rat liver and kidney tissue is difficult to explain solely by the fact that carnitine is involved in fatty acid oxidation (32). Numerous recent reports have suggested roles for carnitine beyond that of long chain fatty acid transport. These functions, such as the buffering of the coenzyme A pool (10), the formation of acylcarnitines involved in branched chain amino acid metabolism (21), and carnitine-mediated transfer of acetyl groups out of the mitochondria have been demonstrated (31).

In the present study, we investigated the possibility of promoting phospholipid synthesis in fetal lungs by means of maternal carnitine treatment. Further, the effects of carnitine and betamethasone on DPPC content and PC species composition in fetal rat lungs were compared.

MATERIALS AND METHODS

We chose the following experimental model. Thirty gravid Wistar rats of stock Ch bb-Thom with an average weight of 300

g and an expected gestation period of 23 days were randomized, divided into three groups, and treated from the 17th to the 20th day of gestation with 1 ml/day physiologic saline (controls to equalize the trauma), or L-carnitine (10 mg/kg in 1 ml distilled water), or betamethasone (0.3 mg/kg in 1 ml distilled water), administered intraperitoneally. Delivery by cesarean section took place on the 21st day. Laparatomy of the mother animals was performed by means of abdominal longitudinal incision. In the course of the hysterotomy, both uterine cornua were opened along their full length. The fetuses, not allowed to breath, were thoracotomized by means of parasternal incision. The fetal lungs were then grouped according to litter in order to preclude station-related differences.

The fetal lungs were homogenized in 40 volumes of chloroform/methanol (2:1, vol/vol) and extracted overnight under nitrogen. The tissue lipid extracts were washed using the method of Folch *et al.* (13), and the phospholipids were assayed by a modified version of Bartlett's method (7).

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine was assayed as a diacylglycerol trimethylsilyl ether derivative by GLC^2, using 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine serving as the internal standard (29).

Aliquots of the samples were applied as bands on previously cleaned thin layer chromatographic plates. The plates were developed in a chloroform/methanol/1% potassium chloride solution (43:47:4, vol/vol/vol) and dried, and the 3-*sn*-phosphatidylcholine fraction was removed from the plates. The 1,2-diacyl-*sn*-glycerols of 3-*sn*-phosphatidylcholine were obtained by digestion with phospholipase C from *Baccillus cereus*. The TMS ethers of 1,2-diacyl-*sn*-glycerols were prepared by reaction with pyridine/hexamethyldisilazane/chlorotrimethylsilane (12:5:2, vol/vol/vol). The *t*-BDMS ethers of diacylglycerols were prepared by the method described by Myher *et al.* (35) by reaction with *t*-BDMS chloride. The resulting TMS and *t*-BDMS ether solutions were used directly for subsequent GLC^2 analyses. The individual diacylglycerol species were then identified by GLC^2-MS combination on the basis of *t*-BDMS derivatives (36).

PC fatty acids were determined as methyl ester derivatives by GLC^2.

Carnitine assay. The tissue was flash-frozen immediately after removal. The perchloric acid extracts were used for assaying the free and short chain acylcarnitine. The carnitine esters were saponified and assayed as free carnitine by radioenzymatic means (34). By using HEPES instead of Tris buffer (11), the determination accuracy was increased.

Chemicals. Chloroform, methanol, pyridine, hexamethyldisilazane, and thin layer chromatographic plates (silica gel 60) were obtained from E. Merck (Darmstadt, F. R. G.). *B. cereus*-derived phospholipase C was prepared by Boehringer (Mannheim, F. R. G.). *t*-BDMS chloride was obtained from Petrarch Systems Inc. (Levittown, PA). Dimyristoyl-*sn*-glycero-3-phosphocholine was supplied by Sigma (St. Louis, MO).

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. Following betamethasone treatment, no increase in phospholipid content was found in the fetal lungs as compared to the control group (71 ± 19 *versus* 72 ± 10 mg/g dw; mean ± SD). By contrast, there was an increase to 80 ± 11 mg/g dw following L-carnitine administration (Fig. 1).

Dipalmitoylphosphatidylcholine. Figure 2 shows the DPPC content of the fetal lungs in mg/g dw and the portion of DPPC in the PC species. Both betamethasone and L-carnitine treatment induced a quantitative elevation of the DPPC content (8.1 ± 2.0 mg/g dw in the betamethasone group; 7.7 ± 2.1 mg/g dw in the carnitine group *versus* 7.0 ± 2.5 mg/g dw in the control group). The portion of DPPC in the PC species exhibited a rise only in the betamethasone group (34.2 ± 3.3%) *versus* the control value

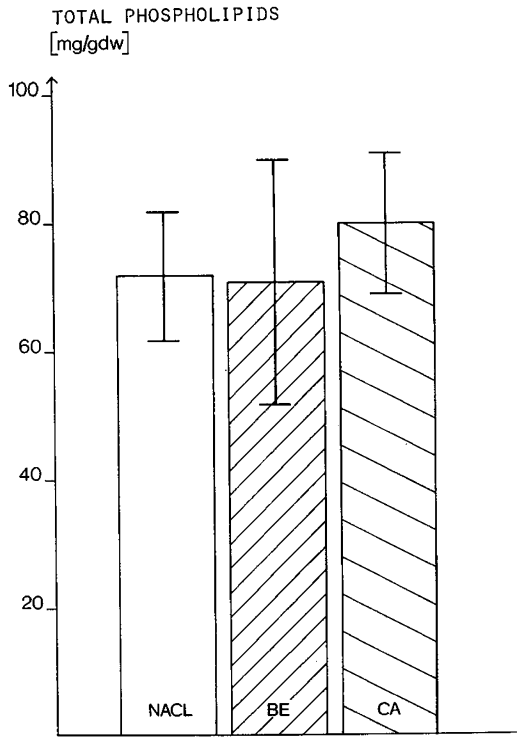

Fig. 1. Total phospholipid content in fetal rat lungs following maternal betamethasone (*BE*) (72 ± 10 mg/g dw; $n = 7$) and L-carnitine (*CA*) (80 ± 11 mg/g dw; $n = 7$) treatment, in comparison to the control group (*NACL*) (71 ± 19 mg/g dw; $n = 7$). *Bars* indicate the mean ± SD in each group.

(27.6 ± 4.5%) ($p < 0.01$), whereas no response to carnitine administration was noted (27.6 ± 2.0%).

These findings indicate that steroid treatment leads to a selective elevation of the DPPC level (per g dw) in the fetal lungs whereas the rise in the DPPC level after carnitine administration appears to be attributable to an overall enhancement of PC content in the fetal lungs.

PC-32 monoenic species. The PC-32 monoenic species can be characterized as 16:0/16:1-PC and 16:1/16:0-PC (29, 36). A relatively high portion of 16:1/16:0-PC is probably characteristic for the fetal lung as compared to the adult lung, since analogous patterns have also been found in human amniotic fluid (30) and fetal sheep lungs (A. Lohninger, unpublished results). Figure 3*A* presents the percentage portion of the 16:0/16:1-PC and Figure 3*B* that of the 16:1/16:0-PC in the PC species of the fetal lungs. 16:0/16:1-PC is significantly ($p < 0.01$) lower in the steroid-treated group (3.6 ± 0.4%) than in the carnitine group (4.4 ± 0.4%) and the controls (4.4 ± 0.4%), whereas 16:1/16:0-PC is present in higher levels in the carnitine-treated group (13.0 ± 3.0%) than in the steroid (10.0 ± 1.1%) and control (11.8 ± 3.3%) groups. These differences in PC species composition are also manifested in quantitative terms (Fig. 4). These findings were confirmed by analysis of the PC fatty acid composition.

Composition of the PC fatty acids. Table 1 shows the relative composition of the esterified fatty acids in the PC of the fetal lungs. The portion of palmitic acid was nearly unchanged in the treated groups in comparison to the control group. The administration of betamethasone resulted in a lowering of the relative portion of the monoenic fatty acids (16:1, 18:1) and to an increase in the relative portion of polyenic fatty acids (18:2, 20:4) in the esterified fatty acids of the pulmonary PC (Table 1).

In comparing the PC fatty acid profile (Table 1) with the changes in the portion of the PC-32 monoenic species in the total PC species after betamethasone treatment (Fig. 3), there is a significant percentage decrease ($p < 0.01$) of 16:1 in the PC fatty acids that coincides with the reduction in the 16:0/16:1-PC

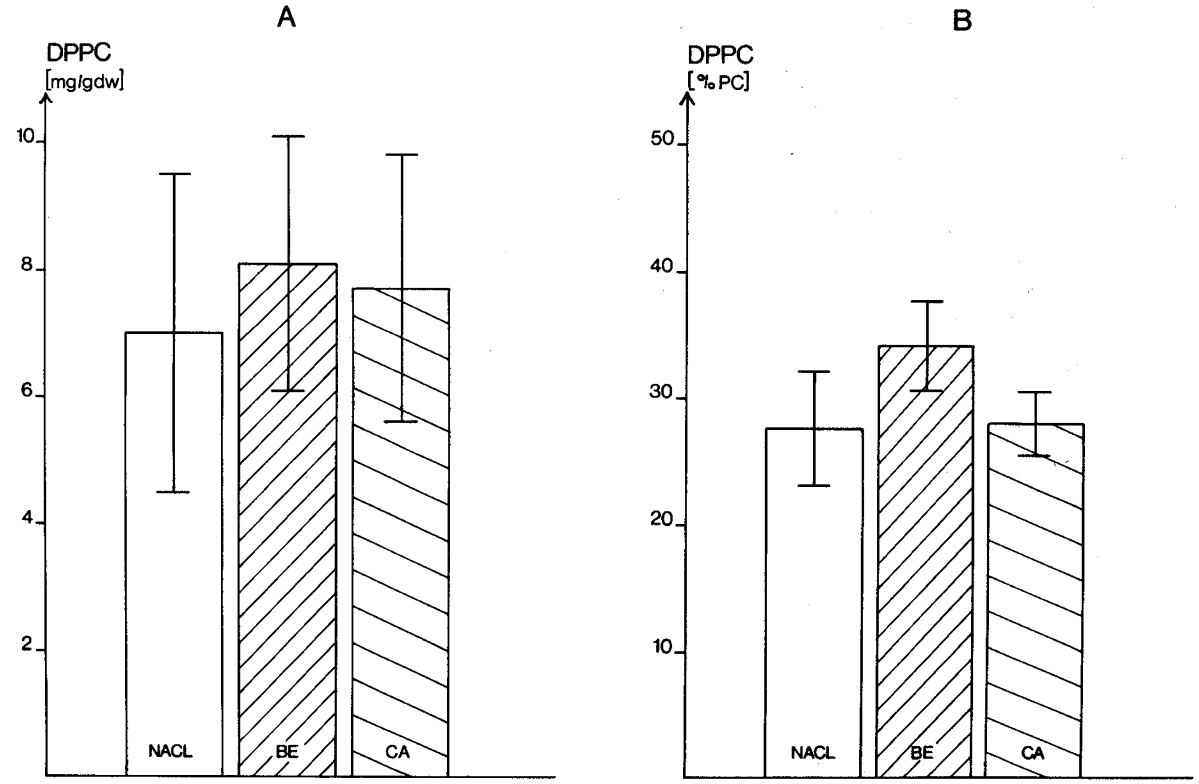

Fig. 2. DPPC content in fetal rat lungs following maternal betamethasone (*BE*) and L-carnitine (*CA*) treatment in comparison to the control group (*NACL*). *A*, DPPC content in mg/g dw (*BE*: 8.1 ± 2.0, $n = 7$; *CA*: 7.7 ± 2.1, $n = 7$; *NACL*: 7.0 ± 2.5, $n = 7$). *B*, portion of DPPC in the fetal lung species (*BE*: 34.2% ± 3.3, $n = 10$; *CA*: 27.7% ± 2.6, $n = 9$; *NACL*: 27.6% ± 4.5, $n = 7$); the *BE* value is significantly ($p < 0.01$) higher compared to those of *NACL* and *CA*. *Bars* indicate the mean ± SD in each group.

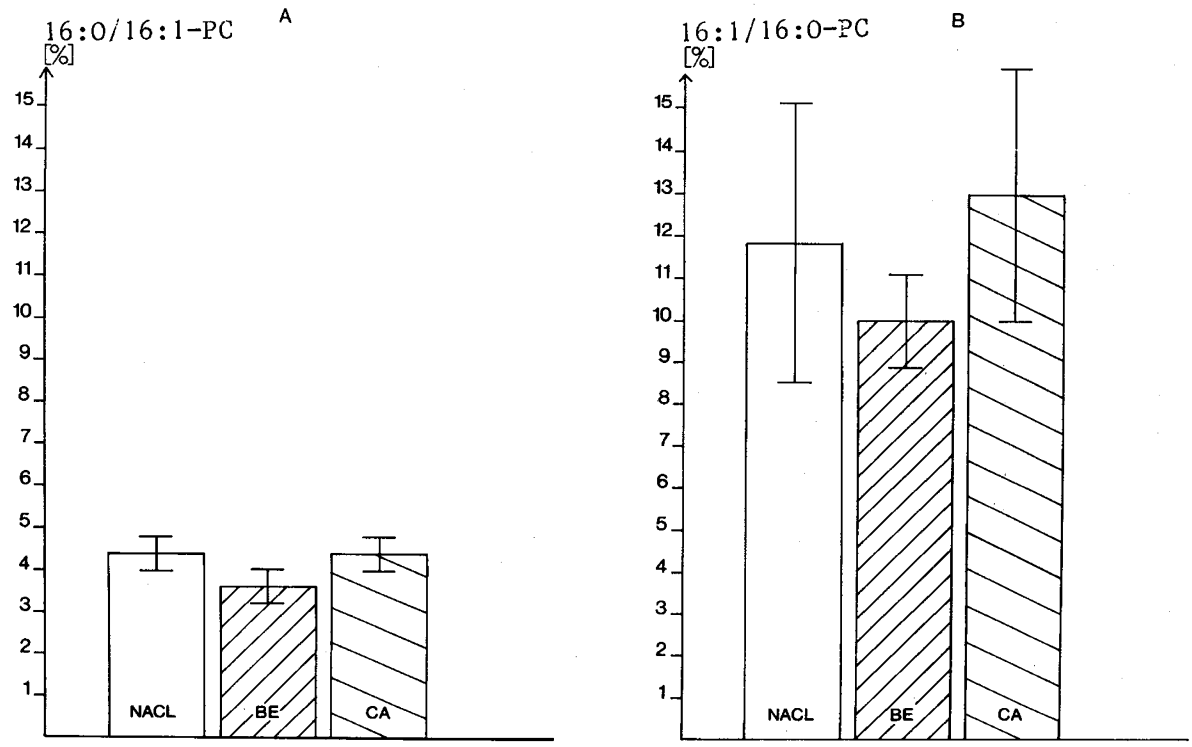

Fig. 3. Portion of the monounsaturated PC-32 (*i.e.* the sum of carbon atoms in the acyl radicals is 32) species in fetal rat lung PC following maternal betamethasone (*BE*) and L-carnitine (*CA*) treatment in comparison to the control group (*NACL*). The PC-32 monoenic species can be characterized as palmitoyl-palmitoleyl-PC (16:0/16:1-PC) (*A*) and palmitoleyl-palmitoyl-PC (16:1/16:0-PC) (*B*). *A*, *BE* (3.6% ± 0.4, $n = 10$) is significantly decreased ($p < 0.01$) compared to *CA* (4.4% ± 0.4, $n = 9$) and *NACL* (4.4% ± 0.4, $n = 7$). *B*, *BE*: 10.0% ± 1.1, $n = 10$; *CA*: 13.0% ± 3.0, $n = 9$; *NACL*: 11.8% ± 3.3, $n = 7$. *Bars* indicate the mean ± SD in each group.

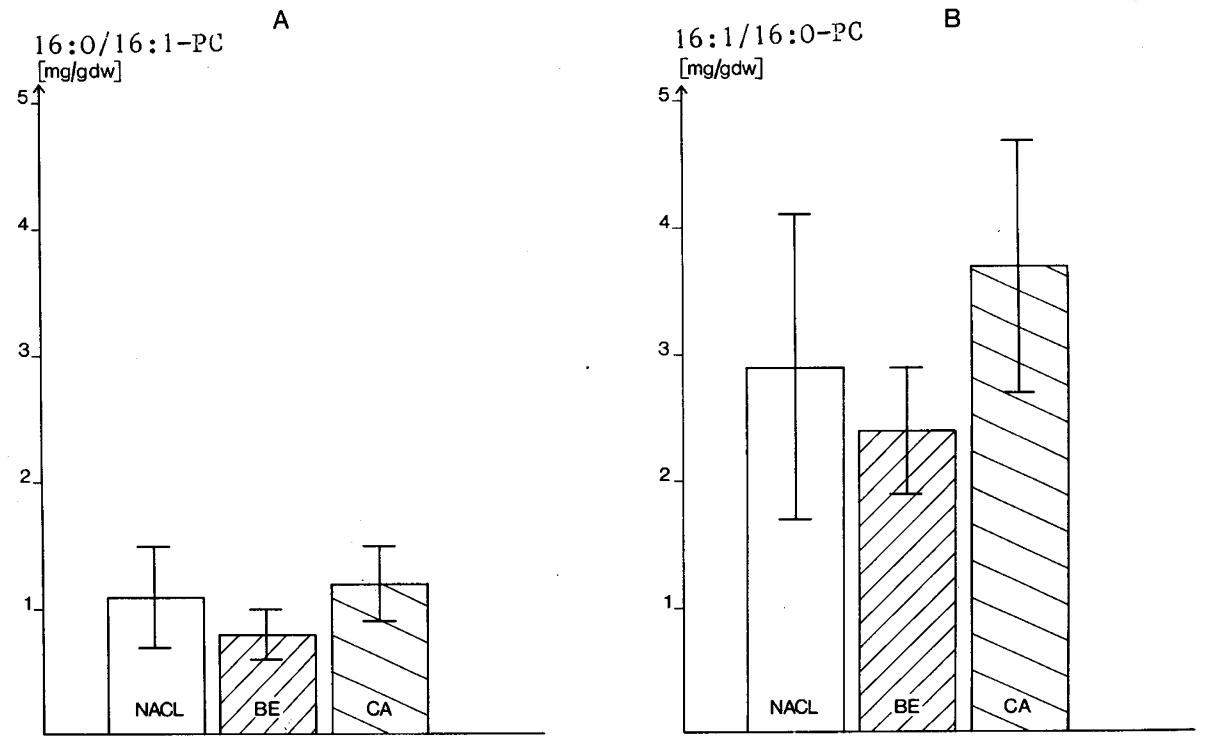

Fig. 4. Absolute content of monounsaturated PC-32 (*i.e.* the sum of carbon atoms in the acyl radicals is 32) species in mg/g dw of fetal rat lungs following maternal betamethasone (*BE*) and L-carnitine (*CA*) treatment in comparison to the control group (*NACL*). The PC-32 monoenic species can be characterized as palmitoyl-palmitoleyl-PC (16:0/16:1-PC) (*A*) and palmitoleyl-palmitoyl-PC (16:1/16:0-PC) (*B*). *A*, *NACL*: 1.1 mg/g dw ± 0.4 ($n = 7$); *BE*: 0.8 ± 0.2 ($n = 7$); and *CA*: 1.2 ± 0.3 ($n = 7$). *B*, *NACL*: 2.9 mg/g dw ± 1.2 ($n = 7$); *BE*: 2.4 ± 0.5 ($n = 7$); and *CA*: 3.7 ± 1.0 ($n = 7$). There is a significant difference ($p < 0.05$) comparing the *BE* group with the *CA* group, in both *A* and *B*. *Bars* indicate the mean ± SD in each group.

Table 1. *Relative composition of esterified PC fatty acids**

Treatment	16:0	16:1	18:0	18:1	18:2	20:4
NaCl (controls) (7)	43.9 ± 2.6	9.1 ± 1.6	8.3 ± 1.7	19.7 ± 2.3	5.5 ± 0.7	6.6 ± 0.7
Betamethasone (11)	45.9 ± 3.2	7.1 ± 1.0†	9.0 ± 1.6	16.9 ± 1.9‡	6.6 ± 0.7	8.5 ± 1.9
Carnitine (10)	44.2 ± 1.8	9.0 ± 1.5	8.8 ± 1.1	23.1 ± 2.7	4.8 ± 0.9	7.2 ± 1.4

* Values are mean ± SD, with number of experiments in parentheses. 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid.

† Significantly lower ($p < 0.05$) compared to the control and carnitine groups.

‡ Significantly lower ($p < 0.01$) compared to the control and carnitine groups.

Table 2. *PC species (percentage of total PC)**

		PC-32						
Treatment	PC-30	Total	16:0/16:1	16:1/16:0	DPPC	PC-34	PC-36	PC-38
NaCl (controls)	9.5 ± 3.3 (7)	43.8 ± 7.4 (7)	4.4 ± 0.4 (7)	11.8 ± 3.3 (7)	27.6 ± 4.5 (7)	31.4 ± 3.5 (7)	13.3 ± 3.1 (7)	3.5 ± 3.3 (4)
Betamethasone	9.5 ± 1.6 (10)	47.9 ± 4.1 (10)	3.6 ± 0.4† (10)	10.2 ± 0.9 (10)	34.2 ± 3.3‡ (10)	28.0 ± 2.9§ (10)	13.2 ± 3.4 (10)	1.8 ± 1.0 (7)
Carnitine	9.6 ± 2.2 (9)	44.6 ± 5.1 (9)	4.4 ± 0.4 (9)	13.0 ± 3.0 (9)	27.7 ± 2.6 (9)	31.4 ± 2.2 (9)	12.8 ± 2.4 (9)	2.2 ± 2.1 (5)

* Values are mean ± SD, with number of experiments in parentheses.

† Significantly lower ($p < 0.01$) compared to the control and carnitine values.

‡ Significantly higher ($p < 0.01$) compared to the control and carnitine values.

§ Significantly lower ($p < 0.05$) compared to the control and carnitine values.

($p < 0.01$) and 16:1/16:0-PC species (Fig. 3, Table 2). By contrast, the reduction of the 18:1 portion in the PC fatty acids ($p < 0.05$) corresponds to the lowering of the PC-34 portion in the PC species ($p < 0.05$) (Table 2).

Carnitine. Figure 5 shows the content of total carnitine (*A*), free carnitine (*B*), and short chain acylcarnitine (*C*) in the fetal lungs. Betamethasone fails to influence the carnitine content of the fetal lungs. Despite the low carnitine dose used (10 mg/kg),

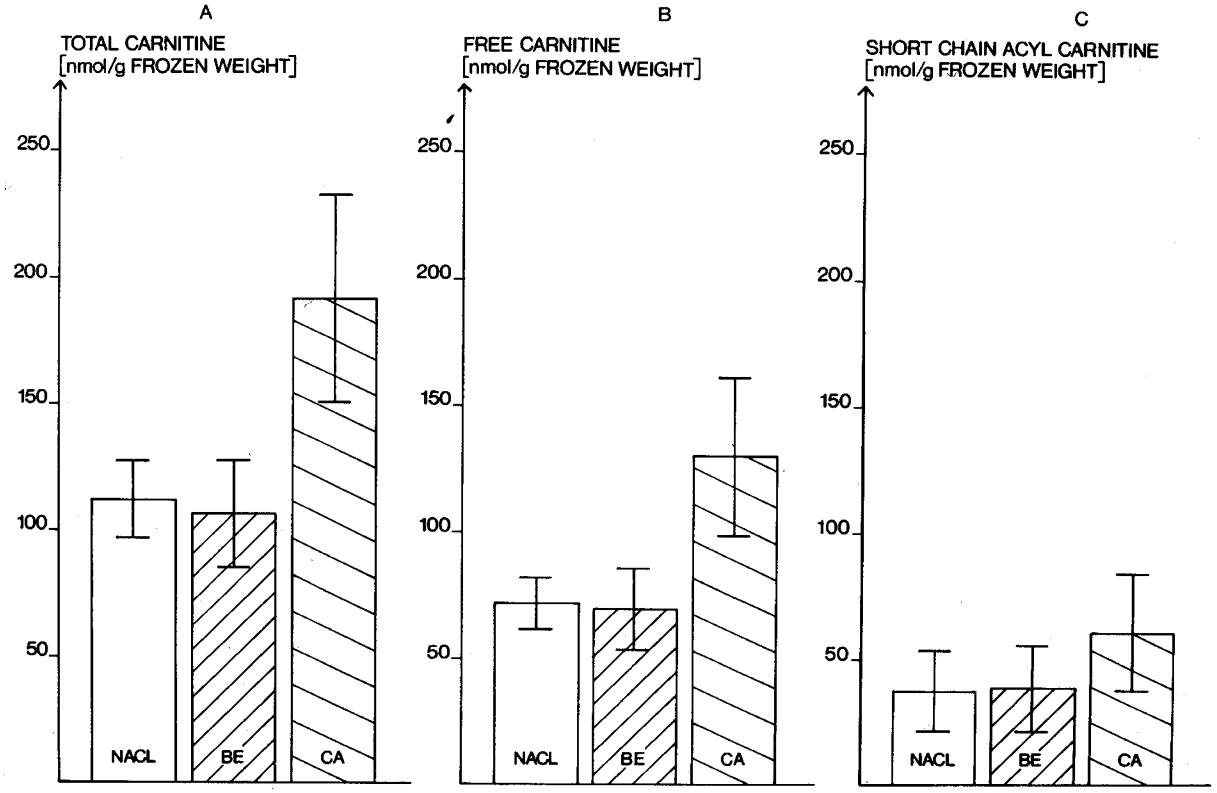

Fig. 5. L-Carnitine content (in nmol/g frozen weight) of fetal rats lungs following maternal betamethasone (*BE*) ($n = 10$) and L-carnitine (*CA*) ($n = 10$) in comparison to the control group (*NACL*) ($n = 6$). *A* shows the content of "total" carnitine (*i.e.* short chain acylcarnitine + free carnitine); the *CA* value, 192 nmol/g frozen weight ± 41, is significantly ($p < 0.01$) higher compared to that of *BE*, 106 ± 21, and *NACL*, 112 ± 15. *B* shows the content of free carnitine; the *CA* value, 130 ± 31, is significantly higher compared to that of *BE*, 70 ± 16, and *NACL*, 72 ± 10 ($p < 0.01$). *C* shows the content of short chain acylcarnitine; *CA*: 61 ± 23; *BE*: 39 ± 17; and *NACL* 38 ± 16. *Bars* indicate the mean ± SD in each group.

there is a marked increase in both free ($p < 0.01$) and short chain acylcarnitine in the fetal lungs.

DISCUSSION

Betamethasone. Anderson *et al.* (3) found a marked increase in the uptake of labeled choline in the PC of fetal lungs as compared to the controls only in doses of between 0.8 and 6.4 mg/kg betamethasone (administered maternally on the 20th day of gestation). On the other hand, a dose of 0.4 mg dexamethasone has been shown to depress DNA by 51%, total PC by 28%, and disaturated PC by 33% (15). In the present study, an increase in DPPC content with respect to the dry weight of the fetal lungs and a simultaneous decrease in all assayed PC species as compared to the control values was found after administration of betamethasone to the mother animals. When we compare the composition of the PC species in the fetal lungs, it is striking that the elevation of the DPPC portion in the PC species (from 27.6 to 34.2%) was compensated for by a depression of monoenic species (Fig. 2). These findings were substantiated by determinations of the fatty acid composition (Table 1).

Of special interest regarding the differences of the PC species composition in fetal lungs and the lungs of adult animals are the two monoenic PC-32 species (16:0/16:1-PC and 16:1/16:0-PC). Whereas in the fetal lungs 16:1/16:0-PC makes up the major portion of the PC-32 monoenic fraction, in the lungs of adult male rats this PC species is only a minor constituent and 16:0/16:1-PC represents the dominant fraction of the PC-32 monoenic species (36). There is no notable reversal of the ratio of these two PC-32 monoenic species following betamethasone treatment, as would be expected in the case of accelerated assimilation of the PC profile of the fetal lungs to that of adult animals. On the contrary, a reduction in both absolute and relative terms of both PC-32 monoenic species is observed. The potential importance of these two PC species for the lung function or as a predictor of fetal lung maturity has yet to be established. In addition to effecting changes in the PC species composition of fetal lungs and an accelerating choline uptake in the pulmonary PC in both *in vitro* and *in vivo* models, corticosteroids also exert an influence on protein metabolism. A marked reduction in DNA content per lung has been reported (2, 15, 25), which points to a retardation of pulmonary mitosis, with differentiation of the lung cells being favored. Thus, morphologic changes in the fetal lungs as compared to untreated controls have been found 24–48 h after steroid treatment and have been interpreted as an expression of functional and morphologic maturation of the lungs (12, 27, 37). Of special importance in this context is the proliferation of alveolar type II cells (37, 45, 50), as well as enhanced formation of lamellated corpuscles in type II cells (37, 38, 50). A characteristic of type II cells is the high portion of disaturated PC in the PC species (8, 33). If the number of type II cells increases with respect to the other lung cells, an elevation in the DPPC content, as found in the present experimental series after betamethasone treatment, should be expected. In addition, stimulation of PC synthesis in type II cells by steroids has been reported (2, 40, 43).

A reduction in the number of lung cells in addition to a possible enhancement of PC synthesis in type II cells could account for the findings of the present investigation, *i.e.* total phospholipid content similar to that of the controls with simultaneous elevation of the DPPC content. A direct comparison of the betamethasone-treated group with the control group could be distorted by an inhibitory effect of the steroid on protein synthesis, which, in turn, would alter the dry weight of the fetal lungs as a point of reference.

Carnitine. In the present study, it was demonstrated that the administration of L-carnitine to pregnant rats elevates the levels

of both free carnitine and acetylcarnitine in the fetal lungs to approximately twice those of the controls and the group treated with betamethasone.

In the untreated fetal rat lungs, the carnitine and acetylcarnitine levels increase from the 19th day of gestation up to parturition (A. Lohninger, unpublished results). A comparison of untreated with L-carnitine-treated animals reveals that the maternal administration of a relatively low L-carnitine dose results in a carnitine level in the fetal lungs on the 20th day that is roughly equivalent to that of untreated animals on the 22nd or 23rd day of gestation (term). Since, however, carnitine was given only in low doses (10 mg/kg), it is highly probable that even higher L-carnitine levels than those obtained in the present study could be attained through the administration of higher L-carnitine doses. After all, owing to its low toxicity (47, 51), carnitine doses of 100–150 mg/kg have already been clinically administered (4, 5).

In most of the tissues there is a higher carnitine concentration than in the plasma. An active transport mechanism for the uptake of L-carnitine across a high concentration gradient has been described in human heart cells (6, 9), in isolated liver cells (10, 11), and in kidney (22) and muscle cells (41) of rats. Carnitine is probably also transported across the plasma membrane of lung cells by the same or at least a similar mechanism. Moreover, it is apparent that this carrier system for the uptake of carnitine by lung cells of fetal rats is activated relatively early in the gestation period. The limiting factor for the carnitine concentration in the cells is probably the lower carnitine concentration in the fetal circulation.

Even if placental passage of carnitine in rats is lower than that in other species, *e.g.* guinea pigs (17), there is a demonstrable increase of both free L-carnitine and L-acetylcarnitine levels in fetal rat lungs following the maternal administration of relatively small amounts of carnitine. Hahn *et al.* (16) were able to show that injection of [^{14}C]carnitine into pregnant rats resulted in the appearance of the label in amniotic fluid. It is of interest to note in this context that in man levels of both free and acetylcarnitine have been found to be higher in cord blood than in maternal blood, suggesting placental transfer to be adequate (16, 19). As in rabbits and guinea pigs, there is a high carnitine tissue level even before birth (18); these species are unsuitable as experimental models for studying carnitine metabolism in fetal lungs, since such findings cannot be extended to human fetal lungs.

The increase in the DPPC content following carnitine treatment could also be accounted for by the investigation of Ishidate and Weinhold (23), who found that a further important pathway of DPPC synthesis takes place via *de novo* synthesis of PC through utilization of dipalmitoylglycerol. With this metabolic pathway, an increase in the DPPC content of fetal lungs would be conceivable in the context of an overall rise in PC synthesis without necessarily entailing stimulation of the deacylation-reacylation cycle or the proliferation of type II cells. However, it is generally assumed that this *de novo* PC synthesis makes only a minor contribution to the DPPC content of the lungs (48, 49).

In considering the effect of carnitine treatment on the quantitative changes of various PC species in fetal lungs (Table 2), it is noteworthy that most of the assayed PC species revealed an increase as compared to the controls. In view of the similar composition of the PC species in the fetal lungs of the control group and those of the carnitine-treated group, an overall elevation of the phospholipid and PC content in the fetal lungs is more probable than a specific stimulation of DPPC synthesis.

The findings of the present study suggest that betamethasone and carnitine effect an elevation of the DPPC level in fetal lungs via two dissimilar mechanisms. Maternal treatment with a carnitine-betamethasone combination may therefore be more effective in increasing pulmonary surfactant production than the administration of carnitine or betamethasone alone.

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Food Proteins and Gut Mucosal Barrier. II. Differential Interaction of Cow's Milk Proteins with the Mucous Coat and the Surface Membrane of Adult and Immature Rat Jejunum

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ABSTRACT. Two *in vitro* intestinal models were used to investigate postnatal maturational changes of the gut barrier functions. Microvillus membrane (MVM) preparations were studied for surface binding, and everted gut sacs were studied for mucous coat binding, breakdown and uptake of radioiodinated bovine serum albumin (BSA), and β-lactoglobulin (β-LG). Surface binding of these proteins to MVM was weak and nonspecific. There was more binding of both proteins to immature MVM (BSA: newborns, 2.74 ± 0.52%, adults, 1.08 ± 0.17%, $p < 0.001$; β-LG: newborns, 6.30 ± 0.54%; adults, 2.05 ± 0.07, $p < 0.001$). In contrast to MVM binding characteristics, mucous coat binding of the cow's milk proteins to immature gut sacs was significantly less (BSA: preweanlings, 0.94 ± 0.30 μg ^{125}I-protein Eq/mg mucosal protein; adults, 3.06 ± 0.74, $p < 0.001$; β-LG: preweanlings, 5.61 ± 1.48; adults, 9.83 ± 1.33, $p < 0.001$). Protein binding and uptake were correlated in the immature animals ($r = 0.76$, $p < 0.001$ for BSA and $r = 0.85$, $p < 0.001$ for β-LG). More β-LG was bound and taken up than BSA in the preweanlings ($p < 0.001$). Trichloroacetic acid precipitation studies showed that, even in the immature rats, β-LG was much more readily broken down by mucosa-associated enzymes than BSA. Immature animals showed less protein breakdown than adult controls. Decreased protein breakdown and mucous coat binding as well as increased MVM binding may account for the increased uptake of intact food antigens in the newborn. Differences between increased MVM binding and decreased mucous coat binding of cow's milk proteins are attributed to protective elements such as the mucus layer which is present in the gut sac model but lacking in MVM. (*Pediatr Res* 18:1252–1256, 1984)

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