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Phospholipid and Fatty Acid Metabolism in *Escherichia coli* -On the Effects of Various Metal Compounds-

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*Escherichia coli*의 인지질 및 지방산 대사 -여러 금속화합물의 효과에 관하여-

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국문요약

*Escherichia coli*에 copper chloride 50 ppm, manganese chloride 100 ppm, nickel chloride 100 ppm을 각각 처리하여 세포를 배양하는 동안에 이들 세포에서 일어나는 인지질 생합성 및 지방산 조성의 변화를 대조구와 비교하여 분석하였다.

세포의 생장과 total lipid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, cardiolipin은 대조구에 비해 금속 화합물 처리구에서 저해되었는데 nickel chloride가 가장 큰 억제 효과를 나타내었다. 그러나 phosphatidylinositol은 금속화합물의 영향을 받지 않았다.

인지질 생합성에 이용된 주요 지방산은 대조구는 palmitic acid(평균 25.47%)와 palmitoleic acid(평균 12.27%)가 인지질 생합성에 도입되었고 copper chloride 처리구는 palmitic acid(평균 30.13%)와 stearic acid(평균 9.12%)로 나타났다. manganese chloride 처리구와 nickel chloride 처리구에서는 모두 palmitic acid(평균 24.16%, 평균 21.77%)와 linoleic acid(평균 9.48%, 평균 11.88%)가 인지질 생합성에 이용된 주요 지방산으로 분석되었다.

Keywords : *E. coli*, phospholipid, fatty acid, metal compounds

I. Introduction

Heavy metals existing as ionized form in water not only cause the pollution of marine ecosystem including algae but also affect photosynthesis of higher plants by absorbing these heavy metals with water(Chun *et al*, 1993).

Because heavy metals were inhibited ATP reduction as blocking electron transfer in oxidative phosphorylation, biosynthesis of DNA and RNA were decreased depending upon deficiency of energy and inorganic phosphate supply and also the formation of inorganic polyphosphate was hindered(Lee and Lim, 1982).

When chloroplast was treated with heavy metals such as manganese, copper, zinc, cobalt and nickel, etc, constriction of chloroplast was observed as it was shown at acidic pH condition (Dilley and Rothstein, 1967).

Copper and nickel were completely inhibited glucose fermentation of *Saccharomyces cerevisiae* (Van Steininck and Booij ,1964). Oleic acid was decreased 27% while arachidonic acid and docosahexaenoic acid were increased 35% and 94%, respectively in fatty acid composed of phospholipid in mouse serum which was fed with low concentration of copper for 11 weeks (Cuannane, 1983).

When *Cunninghamella blakesleeana* was culture in the medium containing copper, the con-

tent of phosphate in cell wall was increased two volumes while that of hexosamine was decreased. And it was also found that hydroxyprolin was specifically shown and citrulline increased abnormally(Venkateswerlu and Stotzky, 1986).

Manganese and nickel induced the abnormal mitosis of *Vicia faba* root(Umeda and Nishimura, 1979; Leonard *et al*, 1981).

Nickel absorbed into sweat gland and hair-follicle ostia have special affinity to keratin(Samitz and Katz, 1976) and so produced tumor along with cobalt and cadimium at certain conditions (Paton and Allison, 1972).

Lipid in organells is related respiration, energy transport, and photosynthesis and the activities of enzymes participated in TCA pathway and respiratory chain are accelerated (Radwan and Mangold, 1976).

Phospholipids composed of biomembranes are cardiclipin(CL), phosphatidylethanolamine(PE), phosphatidylcholine(PC), phosphatidylinositol (PI), phosphatidylglycerol(PG), phosphatidylserine(PS), and phosphatidic acid(PA) etc. (Matsuzake *et al*, 1983). Although the content and type of phospholipid in microorganisms are variant depending upon the strain, PE is predominantly much in the bacterial cells in general (Cronan and Vagelos, 1972). PC was found to be major phospholipid in eucaryotic cells, and phoapholipid composition in mitochondra of mammalia and yeasts were resembled and mainly composed of CL and PE, of which over 90% of CL were existing in inner membrane of mitochondria(Jakovcic *et al*, 1971).

The composition and content of phospholipids in microbial membrane are not only affected by environmental changes such as temperature, pH, medium composition and culture phase (Knivett and Cullen, 1965) but also the catalytic function of enzyme complex of phospholipids as membranous substrate could modify(Stadtlander, *et al*, 1982). When *Pseudomonas aeroginosa* and *E. coli* were treated with polymyxin B, activity of phospholipase was increased and so PE and PG were decreased to 60% and 70%, respectively. The cell viability of *Zymomonas mobilis* was proportionally decreased as the content of ethanol was increased in culture media while vaccenic acid was increased. The concentration of PG against total phospholipid was increased in *Klebisiella aerogenes* treated with cadmium during the culture. On the contrary, it was observed that the production of lypophosphatidylcholin and PS were evident in the minimum medium treated with cadmium and synthesis of PE during the lag phase(12h, 24h) was arisen(Lee *et al*, 1990)

This study were analysed to compare with the control the effect of heavy metals such as copper, nickel, and manganese, etc. in biosynthesis of phospholipid and composition and content of fatty acid.

II. Materials and methods

1. Cell culture

The *E. coli* ATCC 25922 strain used in this work. This strain precultured on agar plates for 24h at 37℃. The precultured cells were inoculated in nutrient broth treated with copper chloride(50 ppm), manganese chloride (100 ppm), nickel chloride 100 ppm, respectively and shaking cultured for 24hr at 37℃ (130 rpm).

2. Extraction of total lipid

Total lipids in cells harvested at the begining and middle phase of the culture were extracted according to the modified method described by Bligh and Dyer(1959). After cells were added chloroform-methanol(1:2,vol/vol) and shaked for 30 min, added the same quantity of distilled water, seperated after leaving and total lipid extracted by filtering the separated chloroform layer using Whatmann No. 1 filter paper.

After methanol layer, upper layer, was added and mixed the same quantity of chloroform, to-

tal lipid reextracted in the separated chloroform layer by filtration using the same filter paper. After the extracted total lipid dried in 40-50℃, dry weight was measured.

3. Seperation and identification of phospholipid

The major phospholipid, such as phosphatidylcholine(PC), phosphatidylethanolamine(PE), phosphatidylinositol(PI),phosphatidylglycerol (PG), and cardiolipin(CL) in the extracted total lipid were separated by thin layer chromatography(TLC, Desaga) according to the procedure of Turner and Rouser(1970). Glass plate(20 X 20cm) used in TLC was precoated with 0.25mm layer of silica gel(Merck), dried in room temperature and activated in 110~120℃ dry oven for 60min before the use. The solvent contained chloroform-methanol-28% ammonia water(65:25:2, v/v/v) for first expansion and chloroform-aceton-methanol-acetic acid-distilled water (3:4:1:1:0.5, v/v/v/v/v) for second expansion were used according to two-one dimention method.

The phospholipid separated from total lipid were identified to compare with the standard materials(Sigma). Developing reagents were used 0.2% ninhydrin in saturated butanol for PE, dragendroff for PC, periodate-shiff's for PI, 20% sulfuric acid with ethanol for PG and CL (Skipski and Ballay, 1969).

4. Methyl esterification of fatty acid

In order to analyze composition and contents of fatty acids composed of phospholipids , PC, PE, PG, PI, CL were methylesterified by Allen and Good method(1971).

Seperated phospholipids in each plate were added 5 m*l* methanol with 5% sulfuric acid and heptadecanoic acid as internal standard, cooled after leaved for 120 min in 68~70℃ dry oven,. and then the same quantity of distilled water was added and shaked. The homogerate was added 2 m*l* hexane and separated hexane layer was added 5 m*l* saturated sodium bicarbonate and separated hexane layer after shaked.

The contents of fatty acid methyl ester in each phospholipid were measured after separated hexane layer dried.

5. Analysis of fatty acid composition

The composition and contents of fatty acids were analyzed by gas chromatograph(GC, Varion 3380). The identification of each fatty acids were resolved by comparision with standard materials(Sigma), such as lauric acid(12:0) and myristic acid(14:0), and palmitic acid(12:0) and palmitoleic acid (16:1), stearic acid(18:0), and oleic acid(18:1), linoleic acid(18:2), linolenic acid(18:3).

The used column, such as stainless steel column(3 mm×3 m) was, used 15% DEGS (diethylglycol succinate) as packing material and H_2-flame ionization detector(FID) as GC detector.

Analysis conditions described as following:

Injection port temperature : 230℃

Column temperature: 170℃

Detector oven temperature: 250℃

Carrier gas :N_2(30 m*l*/min)

III. Result

1. Growth

The growth of *E. coli* treated with copper chloride, manganese chloride, nickel chloride during the culture was shown in Fig.1. The growth in metal compound treatment was inhibited to compare with the control. The average inhibition ratio of growth during the culture was showed 14.05% in copperchloride, 7.43% in manganese chloride, 39.29% in nickel chloride.

2. Total lipid

The changes in content of total lipid in *E. coli* treated with various metal compounds were represented in Fig.2.

The contents of total lipid in copper chloride,

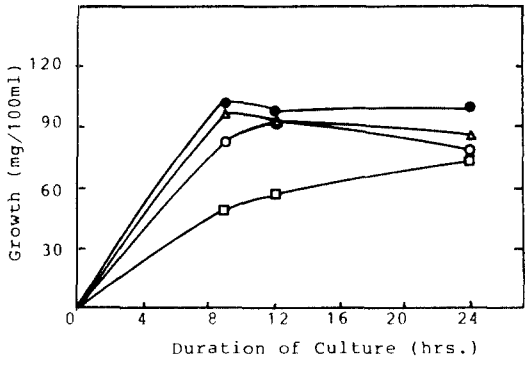

Fig. 1. Effects of metal compounds on the growth of *E. coli* during the cultivation
● Control, ○ Copper chloride
△ Manganese chloride □, Nickel chloride

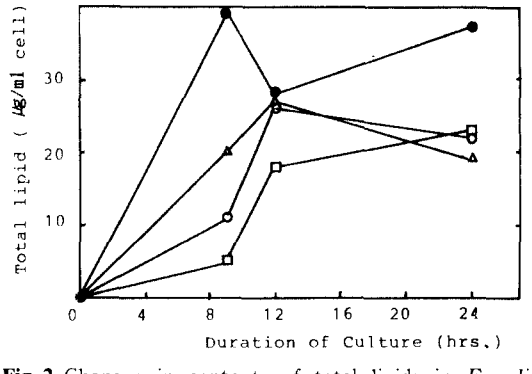

Fig. 2. Changes in contents of total lipids in *E. coli* treated with metal compounds during the cultivation
● Control, ○ Copper chloride
△ Manganese chloride □, Nickel chloride

manganese chloride, nickel chloride were decreased aver. 39.82%, 33.65%, and 52.23%, to compare with the control, respectively.

Nickel chloride and copper chloride treatment remarkably were inhibited 86.15% and 71.79% in comparison with the control in begining of the culture. Manganese chloride tratment was showed the resemble content in the middle of the culture to compare with the control, but decreased 48.65% in the metaphase of the culture.

3. Total fatty acid methyl esters

The changes in contents of total fatty acid

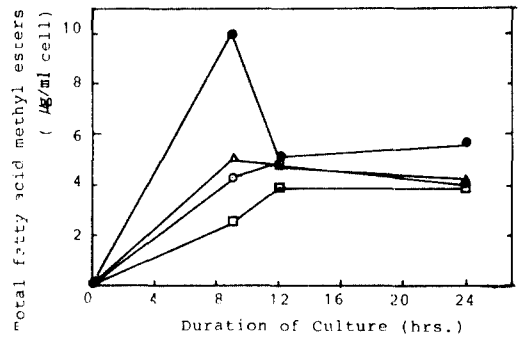

Fig. 3. Changes in contents of total fatty acid methyl esters in *E. coli* treated with metal compounds during the cultivation
● Control, ○ Coppper chloride
△ Manganese chloride □, Nickel chloride

methyl ester in each treatment were recorded in Fig.3. As shown with Fig. 3, the average decreased ratios of total fatty acid methyl esters in copper chloride, manganese chloride, nickel chloride were 26.15%, 24.84%, and 40.14%, respectively.

4. Phospholipid

The contents of various phospholipid in each treatment were represented in Fig. 4 and Fig. 5.

The phospholipids among total lipid in the control were contained 7.57% PE, 1.37% PC, 2.15% PI, 3.14% PG and 4.61% CL. As showed with Fig. 4, PE was decreased aver. 31.12% in copper chloride treatment, aver. 50.16% in manganese chloride treatment, aver. 50.36% in nickel chloride treatment.

PC was inhibited aver. 19.60% in copper chloride treatment, aver. 8.18% in managanese chloride treatment, aver. 26.67% in nickel chloride treatment to compare with the control during the culture.

PI was increased to the middle of the culture in each treatment, but decreased 41.41% in copper chloride treatment, 26.26% in managanese chloride treatment, and 34.34% in nickel chloride treatment at the metaphase of the culture.

PG was decreased 3.87% in copper chloride

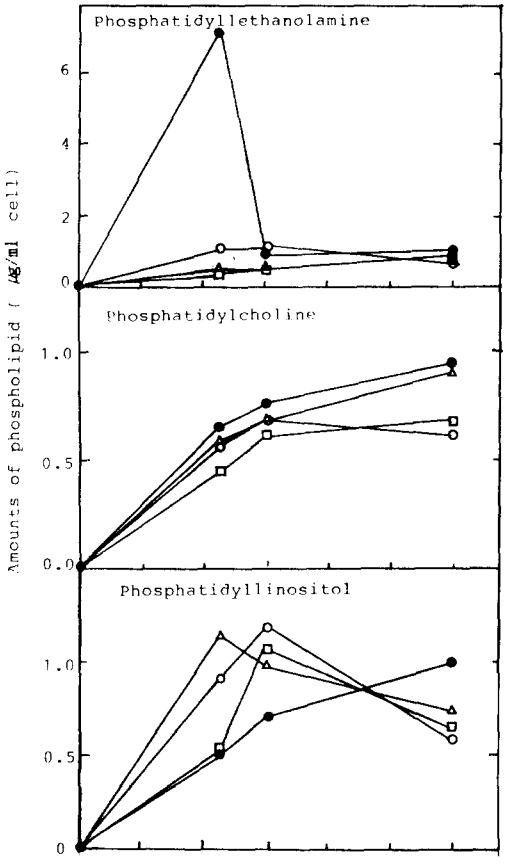

Fig. 4. Changes in contents of various phospholipid in *E. coli* treated with metal compounds during
● Control, ○ Copper chloride
△ Manganese chloride □, Nickel chloride

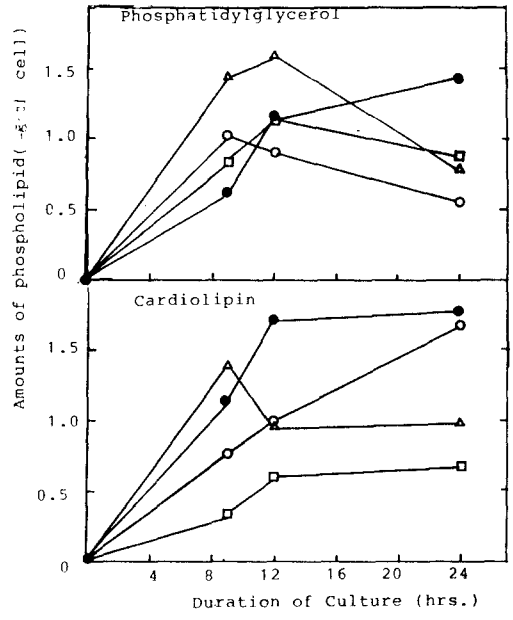

Fig. 5. Changes in contents of various phospholiped in *E. coli* treated with metal compounds during the cultivation.
● Control, ○ Copper chloride
△ Manganese chloride □, Nickel chloride

treatment and showed a resemblance of the conrol in nickel chloride treatment, but increased 10.85% in managanese chloride treatment.

CL was decreased aver. 26.90% in copper chloride, aver. 21.97% in managanese chloride teatment, aver. 66.37% in nickel chloride treatment to compare with the control.

5. Fatty acid

Lauric acid(12:0), myristic acid(14:0), palmitic acid(16:0), palmitoleic acid(16:1), stearic acid(18:0), oleic acid (18:1), linolenic acid(18:2), linolenic acid(18:3) used to phospholipid formation were analyzed.

The contents of fatty acids composed of various phospholipid were showed to Table 1 ~ Table 5.

The major fatty acids composed of PE in the control were used to palmitoleic acid and linolenic acid in 9 hrs. of the culture, and palmitic acid, stearic acid and oleic acid in 12 hrs. and palmitic acid and oleic acid in 24 hrs. The fatty acids in copper chloride treatment were used to palmic acid and stearic acid in 9hrs. and palmitic acid and oleic acid in 12 hrs. and 24 hrs.

The fatty acids constituted in PE in manganese chloride treatment were used to palmitic acid and palmitoleic acid in 9 hrs. and palmitic acid and palmitoleic acid in 12 hrs. and palmitic acid and oleic acid in 24 hrs.

The major fatty acids composed of PE in nickel chloride treatment were palmitoleic acid and stearic acid in 9 hrs. and palmitic acid and stearic acid in 12 hrs. and palmitic acid and palmitoleic acid in 24 hrs.

The fatty acids composed of PC were used to

Table 1. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *E. coli* treated with various metal compounds during the cultivation

Duration of culture (hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid(12:0)	1.17	8.10	0.53	0.69	1.11	1.13	1.37	0.60	5.72	0.79	2.25	0.14	0.80
Myristic acid(14:0)	3.53	11.19	2.22	1.74	1.42	1.94	2.98	3.27	7.19	2.77	2.89	1.37	5.19
Palmitic acid(16:0)	24.49	2.76	31.26	21.37	17.25	20.29	30.18	20.12	47.73	34.19	28.96	29.29	32.88
Palmitoleic acid(16:1)	16.65	16.33	9.35	12.04	21.57	7.52	12.39	8.74	3.94	16.46	14.97	6.62	9.44
Stearic acid(18:0)	3.47	-	18.98	4.00	30.56	10.29	2.36	6.96	12.40	2.00	2.50	5.76	2.83
Oleic acid(18:1)	15.09	1.60	8.39	9.77	-	10.13	14.31	7.29	-	18.40	15.25	14.64	8.62
Linoleic acid(18:2)	3.98	-	9.27	3.10	10.87	8.54	2.99	6.14	10.89	5.40	4.38	5.25	3.06
Linolenic acid(18:3)	3.57	12.94	-	3.11	3.06	5.75	1.06	2.62	-		1.44	-	-
Unknown	23.05	47.08	20.00	44.18	14.16	34.41	32.36	44.26	12.13	trace	27.36	36.94	37.18
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : % Cont : Control

Table 2. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *E. coli* treated with various metal compounds during the cultivation

Duration of culture (hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid(12:0)	0.68	0.32	0.38	0.38	6.36	0.44	0.34	3.54	3.13	0.32	0.68	0.55	0.76
Myristic acid(14:0)	5.19	0.18	1.27	1.01	2.73	2.01	2.76	1.37	4.10	2.30	1.79	2.91	4.49
Palmitic acid(16:0)	26.45	34.52	24.27	28.90	8.67	36.84	35.48	17.69	25.58	32.24	27.86	40.30	31.84
Palmitoleic acid(16:1)	9.72	14.18	5.44	22.14	24.15	15.64	12.50	12.91	0.95	12.86	13.59	13.10	6.71
Stearic acid(18:0)	2.90	2.24	12.68	1.03	5.49	3.64	2.19	3.34	17.33	2.82	4.70	10.79	2.85
Oleic acid(18:1)	13.72	15.92	6.19	15.73	0.12	19.94	14.69	9.35	2.62	16.00	8.83	18.87	8.45
Linoleic acid(18:2)	1.60	1.34	4.16	1.43	10.48	2.58	1.81	8.38	14.68	2.42	5.33	8.23	4.96
Linolenic acid(18:3)	2.43	1.15	-	1.71	5.15	1.19	-	5.12	-	-	0.94	-	-
Unknown	37.31	30.16	45.61	27.67	36.85	17.72	30.23	38.30	31.61	31.04	36.28	5.25	39.94
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : % Cont : Control

palmitic acid and oleic acid in the control during the culture.

The major fatty acids in copper chloride treatment were used to palmitic acid and stearic acid in 9 hrs. of the culture, and palmitic acid and oleic acid in 12 hrs., and palmitic acid and palmitoleic acid in 24 hrs(Table 1).

The main fatty acid used to PC formation in manganese chloride treatment were palmitic acid and palmitoleic acid in 9 hrs. and 12 hrs., and palmitic acid and oleic acid in 24 hrs.

In the event of nickel chloride treatment, the fatty acids used to PC biosynthesis were palmitoleic acid and linolenic acid in 9 hrs. of the culture, and palmitic acid and stearic acid in 12 hrs. and palmitic acid and oleic acid in 24 hrs (Table 2).

The major fatty acid used to PI formation in the control were palmitic acid and palmitoleic acid and myristic acid in 9 hrs. of the culture,

Table 3. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *E. coli* treated with various metal compounds during the cultivation

Duration of culture (hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid(12:0)	1.26	1.65	1.82	8.73	7.78	1.07	0.53	5.01	1.72	3.67	0.86	1.08	1.84
Myristic acid(14:0)	5.20	15.44	4.52	4.54	1.51	4.94	2.38	3.85	3.25	3.89	1.57	4.47	5.00
Palmitic acid(16:0)	5.47	31.66	34.62	21.92	1.91	43.41	29.13	33.26	16.61	38.46	28.22	50.86	33.10
Palmitoleic acid(16:1)	55.86	7.78	0.50	-	21.41	7.53	2.26	-	-	9.26	10.67	2.28	8.15
Stearic acid(18:0)	1.15	2.75	20.47	4.07	5.23	2.51	4.31	7.51	17.06	4.27	5.80	18.90	10.23
Oleic acid(18:1)	3.90	-	-	0.68	-	1.28	4.44	1.42	-	8.31	-		-
Linoleic acid(18:2)	0.47	2.15	10.57	13.66	17.35	7.06	5.50	16.94	26.26	5.91	8.62	13.26	13.38
Linolenic acid(18:3)	2.50	6.54	-	7.90	4.71	4.76	1.24	4.03	-	-	3.95	-	-
Unknown	24.19	32.03	27.50	38.50	40.10	27.44	50.21	27.98	25.10	26. 2	40.31	9.15	28.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : % Cont : Control

Table 4. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *E. coli* treated with various metal compounds during the cultivation

Duration of culture (hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid(12:0)	0.83	1.78	4.33	7.64	6.95	1.91	1.59	5.23	1.78	5.93	1.99	8.3	1.05
Myristic acid(14:0)	5.54	4.52	2.34	16.43	1.51	1.43	3.43	0.16	1.63	0.21	2.53	7.5	4.81
Palmitic acid(16:0)	26.35	30.30	35.94	4.97	1.82	15.99	36.20	5.84	7.72	15.46	37.36	33.2	30.58
Palmitoleic acid(16:1)	9.72	6.69	8.64	4.95	8.46	12.53	10.31	10.13	8.60	13.19	11.12	0.9	1.44
Stearic acid(18:0)	4.50	3.03	19.10	-	13.06	2.63	2.60	14.13	13.09	2.60	5.26	11.5	5.66
Oleic acid(18:1)	3.13	6.19	7.89	0.61	-	8.18	9.25	1.01	-	4.68	4.14	-	3.37
Linoleic acid(18:2)	2.80	2.07	11.48	13.12	12.08	10.34	2.91	12.85	13.20	10.94	5.86	9.6	7.93
Linolenic acid(18:3)	3.10	3.15	-	11.73	10.73	7.90	-	11.09	10.88	trace	3.03	-	-
Unknown	44.03	42.54	10.28	40.55	45.39	39.09	33.71	39.56	43.10	46.99	28.71	28.6	45.16
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : % Cont : Control

and palmitic acid in 12 hrs. and 24 hrs.

The fatty acids composed of PI in copper chloride treatment mainly were used to palmitic acid, stearic acid, linolenic acid, and palmitoleic acid, during the culture. Palmitic acid, linolenic acid, and stearic acid used to PI biosynthesis in manganese chloride treatment.

In the event of nickel chloride treatment, palmitoleic acid, linolenic acid, and stearic acid used to the formation of PI(Table 3).

The main fatty acids constituted in PG in the control were palmitic and palmitoleic acid.

The fatty acids composed of PG in copper chloride treatment were analyzed to palmitic acid, stearic acid, and palmitoleic acid, respectively.

In case of manganese chloride treatment, fatty acids were showed to use myristic acid, linolenic acid, stearic acid, and palmitoleic acid.

It was confirmed that fatty acids in nickel chloride treatment used to stearic acid, , linolen-

Table 5. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *E. coli* treated with various metal compounds during the cultivation

Duration of culture (hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Contl	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid(12:0)	7.46	1.42	6.44	7.44	1.30	6.61	0.32	5.75	1.15	4.84	6.84	1.20	0.47
Myristic acid(14:0)	19.45	16.48	1.64	15.33	0.76	11.03	2.52	2.58	2.86	0.14	3.54	1.78	4.79
Palmitic acid(16:0)	3.27	14.07	24.58	8.31	4.75	17.50	31.06	15.20	28.89	14.66	16.00	31.18	37.14
Palmitoleic acid(16:1)	14.08	14.22	2.94	12.03	18.54	14.61	12.22	8.65	12.91	15.17	2.18	9.11	10.57
Stearic acid(18:0)	-	0.81	26.15	0.58	19.79	0.81	2.86	3.39	6.55	9.50	6.77	11.62	4.71
Oleic acid(18:1)	0.20	1.94	2.75	2.75	-	7.19	13.27	6.57	-	4.15	4.40	9.34	-
Linoleic acid(18:2)	-	-	14.74	10.39	12.73	7.24	3.28	11.06	9.66	10.62	13.52	8.80	8.69
Linolenic acid(18:3)	9.32	7.90	-	9.41	3.37	3.69	0.97	8.42	-	0.56	-	-	-
Unknown	46.24	43.16	20.76	33.76	38.76	31.32	33.50	38.40	39.98	40.36	40.05	26.97	33.63
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : % Cont : Control

ic acid, and palmitic acid(Table 4).

The major fatty acids composed of CL in the control were used to myristic acid, palmitic acid, and palmitoleic acid,

In the event of copper chloride treatment, palmitic acid, stearic acid, oleic acid, and linolenic acid used to the formation of CL.

Myristic acid, palmitoleic acid. palmitic acid, linolenic acid, and stearic acid were used to the biosynthesis of CL in manganese chloride treatment, respectively.

The major fatty acids used to CL formation in nickel chloride treatment, were palmitoleic acid, stearic acid, and palmitic acid (Table 5).

IV. Discussion

Copper plays an important role in a component of plastocyanin participated electron trasport system in chloroplast and a cofactor of various enzyme. Chlorosis in plant arised from due to the inhibition of chloroplast synthesis in the event of copper deficiency(Park and Kown, 1986).

When 1M of nickel was injected into the mouse, it decreased the number of spleen cells producing antibody and also caused abnormality in chromosome of plants and mammalians (Graham *et al*, 1978).

The concentration 10^{-3}M of manganese occurred over 5% of mutation and destroyed cells in metaphases and the above 10^{-3}M of manganese was hindered the biosynthesis of DNA and brought about the abnormality of chromosome (Umeda and Nishmura, 1979).

The growth of *E. coli* treated with copper chloride, manganese chloride and nickel chloride treatment during the culture in this experiment was inhibited by the blocking action of heavy metals.

Mitra *et al*(1975) and Khazaeri and Mitra (1981) reported the mechanism of adaptation of cadmium ion in *E. coli*.

In this experiment, it was thought that the mechanism of adaptation of heavy metals in bacterial cells didn't occurred because of inducement of death phase in copper chloride and manganese chloride treatment beside the growth of control was continued to 24hrs.

But, it was confirmed that *E. coli* was adapted to nickel as a long exponential maintained.

The growth of metal compound treatment were increased at early stage in the culture, but all decreased at later stage. This phenome-

na is because the accumulation of cadimuim was not noticed in early stage but it was very active at later stage.

Therefore, the microbial growth were inhibited due to accumulate copper, manganese and nickel at the later stage in this experiment (Yu *et al*, 1987).

The amino acids converted to keto acid by deamination reaction and acetyl CoA converted through various intermediates used to the synthesis of fatty acid and then the activity of enzyme participated these reaction were blocked by metal compounds.

So not only microbial growth in various metal compounds treatment but also the biosynthesis of total lipid were inhibited.

Not only biosynthesis of phospholipid but also the composition of fatty acids were altered owing to the metal compounds permeated the cells through membrane were inhibited the biosynthesis of nucleic acid and protein.

The biosynthesis of phospholipid and their composition of fatty acids is affected by the environmental factors like temperature, growth, energy metabolism, light and pH etc.(Cronan and Vagelous, 1972) and so the change of phospholipid structure is affected to the fluidity of membrane(Sato *et al*, 1979;Sauer and Heise, 1983)

The composition of fatty acid altered when *E. coli* K-19 were cultured in the media added with methanol and the ratio of saturated fatty acids were decreased and unsaturated fatty acid increased.

Therefore, the fluidity of biomembrane was changed and unsaturated fatty acid were blocked the the infiltration of alcohol into the cells.

Straight-chain fatty acids were found from *E. coli* and *Pseudomonas fluorescens*.

Although it make a difference depending upon the strains, the microbe having straight-chain fatty acids contained the unsaturated fatty(18:1, 18:2) and some saturated fatty acid(16:0)(Kaneda and Smith, 1980).

Gram-negative bacteria specially contained the straight-chain fatty acid and unsaturated fatty acid(Moss *et al*, 1973).

These report accorded this experimental result that palmitic acid and unsaturated fatty acids in the control were confirmed.

And the strains treated with metal compounds were used to unsaturated fatty acid different from the control.

Summary

E. coli was grown as a continous culture at various defined conditions of dilution ratio copper chloride 50 ppm, manganese chloride 100 ppm, and nickel chloride 100 ppm. The investigations had been carried out phospholipid biosynthesis and fatty acid composition changes compared with controls.

Cell growth, total lipid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol and cardiolipin were inhibited when it were treated with heavy metal. Nickel chloride was strong inhibitor but didn't effect on phosphatidylinositol.

Major fatty acid to synthesis phospholipid have analyzed palmitic acid (25.47%) and palmitoleic acid (12.27%) in controls and palmitic acid (30.13%), stearic acid (9.12%) in treated with copper chloride, and palmitic acid (24.16%), linoleic acid (9.48%) in treated with manganese chloride and palmitic acid(21.77%), linoleic acid (11.88%) in treated with nickel chloride.

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