J. KOREAN SOC. FOOD NUTR.
18(1) 62~70

식이중의 Coenzyme Q10첨가가 Adriamycin을 투여한 흰쥐의 심근 미세구조에 미치는 영향

서정숙 · 한인규* · 정형재**

영남대학교 식품영양학과 · *서울대학교 축산학과 · **영남대학교 생물학과

Effects of dietary coenzyme Q10 on adriamycin-induced myocardial ultrastructural changes in rats.

Jung-Sook Seo · In-Kyu Han* · Hyung-Jae Chung**

Dept. of Food and Nutrition, Yeoungnam University, Kyungsan, 713-800, Korea
**Dept. of Animal Sciences, Seoul National University, Suwon, 440-744, Korea*
***Dept. of Biology, Yeoungnam University, Kyungsan, 713-800, Korea*

Abstract

The present study was designed to evaluate whether supplementation of dietary coenzyme Q10 protects the ADR-induced cardiotoxicity in rats. Experiment was undertaken under the condition of simultaneous adminstration of ADR and coenzyme Q10 for 4 weeks. Adriamycin treatment significantly decreased growth performance of rats.But this decrement was not modified by dietary supplementation of conzyme Q10. In the plasma creatine phosphokinase activity, there was no significant difference among experimental groups. Electron microscopic examination revealed a progression of myocardial lesions were dependent upon the level of ADR injection. The most frequently observed fine structural alterations in rat myocardium were mitochondrial swelling, dilation of the sarcoplasmic reticulum and the appearance of a perinuclear vacuolization. But these structural changes were somewhat lesser in defree by dietary supplementation of coenzyme Q10.

INTRODUCTION

Lipid peroxidation in vivo has been identified as a basic deteriorative reaction in cellular mechanisms of aging processes[1]; some phases of atherosclerosis[2]; in chemicals and drugs toxicity[3]; in ethanol-induced injury[4]; in oxygen toxicity[5]. The damaging effects of lipid peroxidation are well documented for mitochondria, microsomes and lysosomes[6]. Lipid peroxidation damage to mitochondria can have profound effects on the cell because lipidperoxidation correlates well with swelling and finally with lysis and disinteg ration of the mitochondria[7].

But cells and tissues are protected against lipid peroxidation damage by a complexity of antioxidant mechanism. Vitamin E may act by scavenging free radicals or as part of membrane structure and thus assist maintenance of the structure and integrity of cell membrane[8]. The

importance of vitamin E in preventing a possible free radical attack on membrane phospholipids has been widely stressed.

Similar antioxidative properties were first ascribed to coenzyme Q by Mellors and Tappel [9] who found that light-induced peroxidation of mitochondrial phospholipids was effectively prevented by coenzyme Q6. In addition, several studies have demonstrated that coenzyme Q in the mitochondrial respiratory chain[10] and the sodium-potassium-activated ATPase of heart microsomes[11] are both inhibited by antitumor antibiotic, adriamycin(ADR). These effects have been evaluated for its potential contribution to the cardiomyopathy. Finally it was reported that the drug appears to induce peroxidation of cardiac lipids in mice by the formation of free radicals, and this effect can apparently be blocked by the concomitant administration of tocopherol[12].

Based on the above background, myocardial ultrastructural changes in rats were observed to evaluate whether coenzyme Q supplementation to animals can protect the ADR-induced cardiotoxicity.

Table 1. Formula and chemical composition of basal diet

Ingredient or chemical composition	content(%)
Ingredient :	
Casein	23.5
Corn starch	40.4
Glucose	11.5
Sucrose	5.8
Butter	5.0
Soubean oil	5.0
α-Cellulose	4.0
Mineral mixture[1]	3.5
Vitamin mixture[2]	1.0
DL-Methionine	0.3
Total	100.0
Chemical composition:	
Crude protein	20.3
	9.3
Total carbohydrate	59.0
Metabolizable energy(kcal / 100 g)[3]	416.0

MATERIALS AND METHODS

Experimental animal and care

Animals used were male rats of Sprague-Dawley strain. Weanling rats were fed a basal diet for 4 weeks until they reached about 250 g body weight. Thereafter, they were assigned to 7 experimental groups of 10 rats and were individually housed in hanging stainless steel cages with wire-mesh bottoms. Rats were fed the experimental diets for 4 weeks. Room temperature was maintained at 20±2℃, with a 12-hour light (08:00-20:00)and 12-hour dark cycle (20:00-08:00). Feed and tap water were provided ad libitum. Except control rats, a dose of 1 or 2mg / kg of body weight of ADR was injected to these animals intraperitoneally (i. p.)on the same day every week. Control rats

[1] The mineral mixture based on the pattern of Rogers and Harper(1965) contained the following (g / 100 g mixture) : $CaCO_3$ 29.29, $CaHPO_4 \cdot 2H_2O$ 0.43, KH_2PO_4 34. 31, NaCl 25.06, $MgSO_4 \cdot 7H_2O$ 9.98, $Fe(C_6H_5O_7) \cdot 6H_2O$ 0.623, $CuSO_4 \cdot 5H_2O$ 0.156, $MnSO_4H_2O$ 0.121, $ZnCl_2$ 0.02, KI 0.005, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0025, $Na_2SeO_3 \cdot 5H_2O$ 0.0015.

[2] 100 g of Vitamin mixture contained the following : Vitamin A acetate 50,000 IU, Vitamin D 10,000 IU, Vitamin E ancetate 500mg, Vitamin K 500mg, Thiamin HCl 120mg, Pyridoxine HCl 800mg, Cyanocobalamin 0.05mg, Ascorbic acid 3,000mg, PABA 500mg, Niacin 600mg, Inositol 600 mg, Choline chloride 20,000mg, Niacin 600mg, Inositol 600mg, Choline chloride 20,000mg, Riboflavin 400mg.

[3] Calculated value.

received 0.9 % saline solution in the same manner as a placebo. Body weight and feed consuption were recorded weekly.

Experimental diets

The composition of basal diet is shown in Table 1. Two experimental diets (A1Q1, A2Q1 group) consisted of basal diet containing 0.1 g coenzyme Q10 per kg of diet. On the other hand, other two experimental diets (A1Q2, A2Q2 group) contained 0.5g coenzyme Q10 per kg of basal diet and the others(control, A1Q0, A2Q0 group) contained the basal diet without coenzyme Q10. Coenzyme Q10 was supplied by Eisai Phamaceutical Company Ltd. (Tokyo, Japan). Fresh diet was provided to the rats daily.

Sample collection

At the end of experimental period, rats were anesthetized with ethyl ether after 16 hour fasting. Blood was collected from abdominal aorta with a heparinized syringe and then centrifuged at 1000 x g for 10 minutes to separate plasma from the cells. The heart was promptly removed, rinsed with 0.02M tris-buffer(pH 7. 4) and blotted in filter paper and weighed.

Determination of creatine phosphokinase activity

The creatine phosphokinase (CPK) activity of plasma was determined by spectrophotometric(UV) method[13]using kits manufactured by Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Absorbance at 340 nm was read at timed intervals of 30 sec for 5 minutes. The activity was then calculated as follows

$$\text{m unit / ml} = \triangle E / \text{min} \times 5{,}000$$

Electron microscopic studies

Morphological study : Tissue was removed from the left lateral lobe of the heart while the animal was under ether anesthesia. Tissue samples were minced into small cubes, fixed in one percent osmium tetraoxide with caccodylate buffer for 1 hour at 4℃ and for 30 minutes at room temperature. And then the tissue was dehydrated in a graded ethanol series and was embedded in Epon 812. The blocked samples were cut on a Porter-Blum MT-2 ultramicrotome with glass knives and then were picked up on uncoated gribs and stained with lead hydroxide. Finally, the sections were examined in a Hitach H-600 electron microscope.

Morphometric study : The morphometric sampling procedure was based on the techniques of Weibel et al[14]. For electron microscopic morphometry, from each group 30 electron micrographs were made at a magnification of 40,000 X. Each density estimation of mitochondria was applied to point-counting method using multipurpose test grid (84 line / 90 x 90.9 mm). The number of points counted by point-counting method was applied to the formula given by Weibel et al[15].

Statistical analyses

In the statistical analysis, the treatment effects were followed by one-way analysis of variance and Duncan's new multiple reange test[16]

RESULTS AND DISCUSSION

Growth Performance

As shown in Table 2, there were significant differences in body weight gain between control and six ADR-treated groups. ADR-treated rats were significantly decreased in the body weight gain compared with control rats. With increasing the ADR-injected level, the weight gain was greatly decreased among experimental groups. This phenomenon was not modified by the

Table 2. Effect of dietary coenzyme Q10 on growth performance of ADR treated rat

Group	Initial body wt. (g)	Final body wt. (g)	Weight gain (g/day)	Feed intake (g/day)	FER
C	246.1±43.9	395.5±73.3[a]	3.94±0.94[a]	22.92±2.73[a]	0.172±0.040[a]
A1Q0	246.2±29.8	354.9±24.7[ab]	2.79±0.53[b]	21.12±1.43[b]	0.132±0.037[ac]
A1Q1	246.2±31.6	349.4±37.5[b]	2.55±0.60[b]	19.65±1.83[cd]	0.130±0.019[ad]
A1Q2	246.3±30.0	353.3±37.7[ab]	2.75±0.28[b]	20.79±0.98[bc]	0.132±0.018[ad]
A2Q0	246.5±27.0	324.2±37.0[b]	1.99±0.62[c]	19.16±1.14[d]	0.104±0.045[bcd]
A2Q1	246.2±25.1	309.3±28.7[b]	1.59±0.37[c]	23.41±1.07[a]	0.068±0.049[b]
A2Q2	246.2±23.8	313.5±37. 4[b]	1.73±0.50[c]	19.62±1.74	0.088±0.018[bd]

[1] Values shown are the meano±S.D.(n=10)

[2] Values with a common superscript letter within the sme column are not significantly different

coenzyme Q10 supplementation. Feed intake was significantly decreased for the experimental groups in comparison with that of control group. However, feed intake of A2Q1 group was not different from that of control group. Feed efficiency ratio was significantly decreased in the groups injected higher dose of ADR, and A2Q1 group showed the lowest value but it was not also influenced by dietary coenzyme Q10.

Present results revealed that ADR treatment had influence on growth performance of rats. The rats injected at higher level of ADR had rapid weight loss compared to control rats. On the other hand, present data indicated that dietary coenzyme Q10 affected slightly the growth rate of rats. Similarly Jaenke[17] reported that rabbits receiving ADR attained less weight gain compared to controls. Fujita et al[18]. also presented that by the repeated treatment of ADR, the body weight of guinea pigs gradually decreased. And he found that ascorbate administration inhibited this decrease in body weight, but did not protect against weight loss by prolonged ADR treatment. The study with human subjects by Ishikawa et al[19]. demonstrated that body weight in patients with congestive heart failure was not changed by coenzyme Q10 supplementation. These results were partially consistent with those of present study.

Effects of dietary coenzyme Q10 on creatine phosphokinase activity in ADR treated rat

Plasma CPK activity was determined as possible biochemical parameter of myocardial damage with the presumption that elevation of CPK reflects myocardial muscle damage[20]. As shown in Table 3, there was no significant difference among experimental groups.

Olson and Capen[21] reported that significant increases of serum CPK in association with myocardial damage after ADR injection were found to precede the onset of highest mortality rate. Jaenke[17] showed that serum CPK was elevated early in the treatment period of all groups of rabbits receiving ADR and continued to rise as treatment continued. However, he stated that in the posttreatment period, CPK values declined rapidly and by the end of the experiment returned to control ranges. This finding would support the report by Preston et al.[22], who claimed that the chief weakness in the response of serum CPK in association with myocardial function

Table 3. Creatine phosphokinase activity in plasma of rats as influenced by ADR treatment and dietary coenzyme Q10

Group	CPK(mUnit / ml plasma)
C	57.98±6.51[a]
A1Q0	71.29±10.89[a]
A1Q1	69.18±19.90[a]
A1Q2	60.37±9.02[a]
A2Q0	70.89±8.43[a]
A2Q1	60.42±5.44[a]
A2Q2	63.48±13.41[a]

1) Vaues shown are the mean±S.D.(n=10)
2) Values with a common superscript letter within the same column are not significantly different ($p<0.05$).

was the fact that elevations following dysfunction were relatively short-lived.

Compared with these reports, the present result was considered to have no difference among experimental groups due to the different experimental condition.

Myocardial ultrastructural changes in rats as influenced by ADR treatment and dietary coenyme Q10

The microscopic features of the myocaridal cellular alteration in ADR and dietay coenzyme Q10-administered rat are presented in Fig. 1. The control rats fed the basal diet showed the well preserved subcellular structure (Fig. 1A). The most frequently observed myocardial alterations in rat which received lower level of ADR alone were dilation of the sarcoplasmic reticulum and the appearance of a perinuclear vacuolization (Fig · 1Ba). In some fields of the cardiac tissues, the characteristic ultrastructural features were obtained: separation of the intercalated discs and mitochondrial swelling: dilation of capillary endothelium (Fig. 1Bb). In the heart tissues from A1Q2 rat which received exogenous coenzyme Q10 , these characteristic alterations seen in ADR-treated animals were not observed. Although there was intracellular edema with mitochondrial proliferation, this change showed the preservation of the myocyte from ADR-induced toxicity (Fig. 1C). The administration of higher dose of ADR resulted in the presence of myelin figures characterized by the ADR-damaged myocytes (Fig. 1D). In the cardiac ultrastructural changes of A2Q2 rats, mitochondria showed condensation of cristae and matrix. Also, occasional vacuoles in the myocyte were seen(Fig. 1E).

The present study established that rat develops

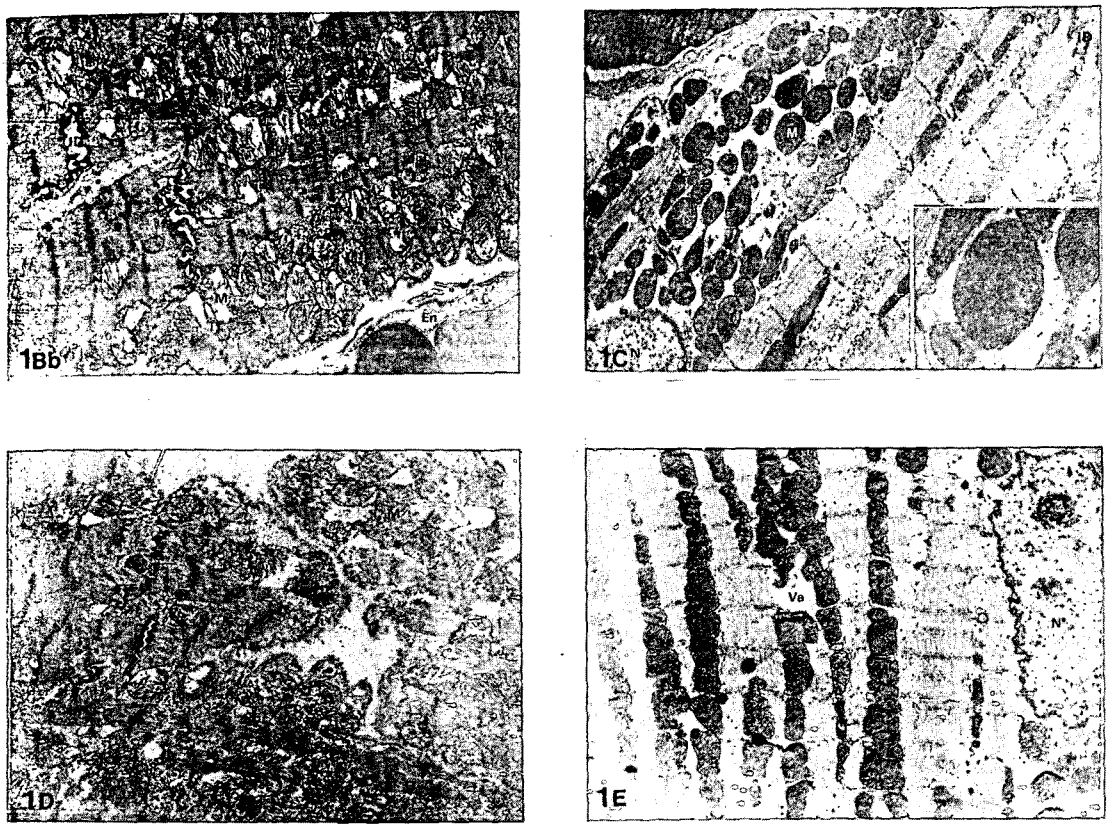

Fig. 1. Transmission electron micrographs of myocardium from simultaneous adminsistation of ADR and coenzyme Q10 (x 12.000).

A. Control group ; Myofibers contain straplike myofibrils with prominent Z bands at regular intervals. Mitochondria (M) are interspersed in regular rows between the fibrils. N ; Nucleus, Z ; Z-band, SR ; Sarcoplasmic reticulum, T ; t-tuble, C ; Capillary, En ; Endothelium.

B. A1Q0 group ; (a). Prominent vacuolation(Va) is present around nucleus and slight dilation of sarcoplasmic reticulum (SR) is seen.
(b). There is marked separation of the interclaated disc(I.D.) between myocytes. A convoluted memtochondria (M) are severely swollen and vacuolated. The capilary is lined by swollen endothelium (Em). R ; RBC.

C. A1Q2 group ; Mitochondrial proliferation is evident. Intercalated dsc(ID) appears intact. Inset x 30,000.
Mitochondria are condensed with numerous cristas (arrow) and matrix.

D. A2Q0 g.oup ; Myelin figures characterized the ADR-damaged myocytes are seen.

E. A2Q2 group ; Mitochondria show condensation of cristae and matrix. Occasional vacuoles in the myocyte are seen.

cardiomyopathy even after lower dose level of ADR administration. The sarcoplasmic vacuolization considered as a prominent sign of ADR-induced myocardiopathy[23] was observed in the heart tissues from A1Q0 rats (Fig. 1Ba), but was not present in those of coenzyme Q10-ad-

Table 4. Characteristic morphometric indices of rat heart mitochondria in Exp. 1

Group	Vv (μm^3)	ISv (μm^2)	OSv (μm^2)	Nv
C	0.427 ± 0.072^{a}	11.4 ± 2.37^{ae}	2.16 ± 0.27^{a}	0.569 ± 0.145^{a}
A1Q0	0.397 ± 0.124^{ac}	11.08 ± 2.85	2.13 ± 0.33^{a}	0.633 ± 0.333^{a}
A1Q1	0.425 ± 0.097^{a}	11.89 ± 2.41^{ae}	2.15 ± 0.48^{a}	0.589 ± 0.108^{a}
A1Q2	0.419 ± 0.074^{a}	13.43 ± 2.79^{b}	2.26 ± 0.54^{a}	0.486 ± 0.169^{a}
A2Q0	0.349 ± 0.071^{bc}	9.51 ± 1.53^{cd}	1.55 ± 0.23^{b}	0.503 ± 0.150^{a}
A2Q1	0.340 ± 0.067^{b}	9.25 ± 1.82^{c}	1.68 ± 0.32^{b}	0.490 ± 0.157^{a}
A2Q2	0.376 ± 0.077^{ab}	12.64 ± 2.30^{be}	2.25 ± 0.39^{a}	0.557 ± 0.184^{a}

1) Vv: Volume density of mitochodria (mitochondrial volume per $1\mu m^3$ tissue)
ISv: Surface density of mitochondrial innermembrane (mitochondrial innermembrane surface per $1\mu m^3$tissue)
OSv: Surface density of mitochondrial outermembrane (mitochondrial outermembrane surface per $1\mu m^3$ tissue)
Nv: Numerical density of mitochodria(mitochondrial number per $1\mu m^3$tissue)

2) Values shown are mean±S.D.(n=30)

3) Values with a common supercript letter within the same column are not significantly different ($p<0.05$).

ministered rats.

The present work was undertaken based on the assumption that, since one possible mechanism of ADR cardiac toxicity may be associated with peroxidation of cardiac lipids, the cardiac toxicity may be reduced by treatment of the animals with coenzyme Q10. In this regard, Myers et al[24]. also reported that prior treatment with tocopherol significantly decreased the ADR-induced cardiomyopathy, and confirmed it by means of electron microscopy. In conclusion, the drug appears to induce peroxidation of cardiac lipids by the formation of free radicals and this effect can apparently be reduced by the concomitant administration of coenzyme Q10.

The tissue contains large numbers of similar bodies, e.g. numerous mitochondria. These bodies take up a characteristic proportion of the cellular volume[14]. The volume density as well as the surface and numerical density of mitochondria were assessed by electron microscope morphometrics as shown in Table 4. Myocardial mitochondria were not generally affected by administration of lower dosage of ADR and dietary coenzyme Q10. With higher dose level of ADR treatment, the numerical density of mitochondria was not much decreased, whereas volume and surface density of mitochondria were greatly decreased. Especially, the surface density of mitochondrial innermembrane considered as an index of oxidative phosphorylation was significantly decreased in higher dose groups of ADR in comparison with that of control group. However, dietary supplementation with higher dosage of coenzyme Q10 restored this decrease. These results indicated that supplementation of coenzyme Q10, a constituent of mitochondrial respiratory chain, increased the volume and surface density of mitochondria, and thereby

preserved the mitochondrial function.

요 약

본 실험은 식이중에 첨가된 coenzyme Q10이 adriamycin (ADR)을 투여한 흰쥐의 심근 미세구조에 미치는 영향을 규명하기 위하여 실시되었다. 실험군은 모두 ADR 2수준(1.0mg/kg B.W./week, 2.0mg/kg B.W./week)과 coenzyme Q10 3수준(무첨가군, 0.1g/kg diet 및 0.5g/kg diet)에 의한 6개의 실험군과 basal diet만을 공급하는 대조군을 설정하였다. 체중증가량은 ADR의 투여수준이 높을수록 유의적으로 감소되었으며 식이효율에 있어서는 고수준의 ADR 투여시에만 대조군에 비하여 감소되었다. 그러나 coenzyme Q10에 의한 회복효과는 나타나지 않았다. 혈장내 CPK 활성도는 ADR투여로 약간 증가되었으나 유의적인 차이는 없었으며 coenzyme Q10 급여에 대한 영향은 나타나지 않았다. 전자현미경을 통해 심장조직의 형태적변화를 살펴본 결과 ADR투여로 인해 심근세포내 미세구조의 변성을 관찰할 수 있었고 특히 mitochondria 의 변형, myofibil을 비롯한 세포 소기관의 소실 및 파괴로 빈 공간이 형성되어 있었으나 coenzyme Q10 급여군에서는 그 정도가 약화되었다. 고수준의 ADR 투여는 mitochondria 의 volume density와 surface density를 크게 감소시켰지만 고수준의 coenzyme Q10 급여에 의해 이러한 감소가 조절되었다.

(본 논문은 1988년도 영남대학교 학술연구비 지원에 의하여 수행되었음).

Reference

1. Csallany, A. S., Ayaz, K. L. and Su, L. C. : Effect of dietary vitamin E and aging on tissue lipofuscin pigment concentration in mice. *J.Nutr.*, 107, 1792(1977)
2. Glavind, J., Hartmann, S., Clemmesen, J., Jensen, K. E. and Dam, H. : Studies on the role of lipid peroxidation in human pathology : 2. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta Pathol. Microbiol. Scand.*, 30, 1(1952)
3. Di Luzio, N. R. : Antioxidants, lipid peroxidation and chemical-induced liver injury. *Fed. Proc.*, 32, 1875(1973)
4. Rosenblum, E., Gavaler, T. S. and van Thiel, D. H. : Lipid peroxidation : A mechanism for ethanol associated testicular injury in rats. *Endocrinology*, 116, 311(1985)
5. Chow, C. K. : Nutritional influence on cellular antioxidant defense systems. *Am. J. Clin. Nutr.*, 32, 1066(1979)
6. Chio, K. S., Reiss, U., Fletcher, B. and Tappel, A. L. : Peroxidation of subcellular organelles ; Formation of lipofuscinlike fluorescent pigments. *Science*, 166, 1535(1969)
7. Hunter, F. E., Scott, A., Hoffsten, P. E., Gebicki, J. M., Weinstein, J. and Schneider, A. : Studies on the mechanism of swelling, lysis and disintegration of isolated liver mitochondria exposed to mixtures of oxidized and reduced glutathione. *J. Biol. Chem.*, 239, 614(1964)
8. Chow, C. K. : Glucose and dietary vitamin E protection against catalase inactivation in the red cells of rats. *Int. J. Vit. Nutr. Res.*, 50, 364(1980)
9. Mellors, A. and Tappel, A. L. : The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J. Biol. Chem., 241,* 4353(1-966)
10. Lenaz, L. and Page, J. A. : Cardiotoxicity of adriamycin and related anthracyclines. *Cancer Treat. Rev.*, 3, 111(1976)
11. Gosalvez, M., Van Rossum, G. V. D. and Blanco, M. F. : Inhibition of sodium-potassium- activated adenosine 5'-triphosphotase and ion transport by adriamycin. *Cancer Res.*, 39, 257(1979)
12. Myers, C. E., McGuire, W. P. and Liss,

R. H. : Adriamycin ; The role of lipid peroxidation in cardiac toxicity and tumor response. *Science,* 197, 165(1977)

13. Tanzer, M. L. and Gilvarg, C. : Creatine and creatine kinase measurement. *J. Biol. Chem.,* 234, 3201(1959)
14. Weibel, E. R., Staubli, W., Gnagi, H. R. and Hess, F. A. : Correlated morphometric and biochemical study on the liver cell. 1. Morphometric model, stereologic methods and normal morphometric data for rat liver. *J. Cell. Biol.,* 42, 68(1969)
15. Weibel, E. R., Kistler, G. S. and Scherle, W. F. : Practical stereological methods for morphometric cytology. *J. Cell Biol.,* 30, 23(1966)
16. Ott, L. : *An introduction to statistical methods and data analysis.* PWS Publishers, Boston., 376(1984)
17. Jaenke, R. S. : Delayed and progressive myocardial lesions after adriamycin administration in the rabbit. *Cancer Res.,* 36, 2958 (1976)
18. Fujita, K., Shinpo, K., Yamada, K., Sato, T., Niimi, H., Shamoto, M., Nagatsu, T., Takeuchi, T. and Umezawa, H. : Reduction of adriamycin toxicity by ascorbate in mice and guinea pigs. *Cancer Res.,* 42, 309(1982)
19. Ishikawa, K., Kanamasa, K., Osato, S., Oda, A., Ogawa, I. and Katori, R. : Increase of cardiac output by Co Q10 in patients with congestive heart failure. In "*Biomedical and Clinical Aspects of Coenzyme Q*", Folkers, K. and Y. Yamamura eds. Elsevier, Amsterdam, N.Y., Oxford. 5, 311, (1986)
20. Bauer, J. D. : *Clinical LaboratoryMethods* 9th ed. The C.V. Mosby Co. 572(1982)
21. Olson, H. M. and Capen, C. C. : Subacute cardiotoxicity of adriamycin in the rat. *Lab. Invest.,* 37, 386(1977)
22. Preston, J. A., Batsatkis, J. G., Briere, R. O. and Taylor, R. V. : Serum creatine phosphokinase, *J. Clin. Pathol.,* 44, 71(1965)
23. Dimitrov, N. V., Hay, M. B., Siew, S., Hudler, D. A., Charamella, L. J. and Ullrey, D. E. : Abrogation of adriamycin-induced cardiotoxicity by selenium in rabbits. *Am. J. Pathol.,* 126, 376(1987)
24. Myers, C. E., McGuire, W. and Young, R. : Adriamycin ; Amelioration of toxicity by α-tocopherol. *Cancer Treat. Rep.,* 60, 961 (1976)

(Received January 16, 1989)