Effect of Erythorbic Acid Administration on Activities of Drug Metabolic Enzyme and Phosphatases in Guinea Pigs Administered an Adequate Amount of Ascorbic Acid

Emiko SUZUKI,¹ Tadao KURATA,² and Nobuhiko ARAKAWA¹

¹ *Department of Food and Nutrition,* ² *Institute of Environmental Science for Human Life, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo 112, Japan*

(Received November 22, 1988)

Summary The effect of erythorbic acid (ErA) administration on activities of liver aniline hydroxylase, liver acid phosphatase, and serum alkaline phosphatase, and the content of liver cytochrome P-450 was studied to determine whether or not ErA would affect the availability of ascorbic acid (AsA) in normal and AsA-deficient guinea pigs. In experiment I, changes of the enzyme activities and liver cytochrome P-450 content in the guinea pigs administered AsA and/or ErA and sacrificed on days 4, 10, 16, and 30 were examined. Moreover, in experiment II, after 16 days of depletion of AsA, the guinea pigs were administered AsA and/or ErA. These animals were sacrificed on days 0, 4, and 20 of the repletion period, after which the activities of drug metabolic enzyme and phosphatases and content of cytochrome P-450 during recovery were observed. The enzyme activities and cytochrome P-450 content of AsA-supplemented guinea pigs were similar to those of ErA-supplemented animals and also similar to those of both AsA and ErA-supplemented guinea pigs throughout the experimental period. During the repletion of the AsA-depleted guinea pigs, there were no significant differences in these enzyme activities and cytochrome P-450 content among the animals administered AsA and/or ErA. These results suggested that ErA administration may not affect the AsA availability in the guinea pigs.

Key Words ascorbic acid, erythorbic acid, guinea pig, aniline hydroxylase, acid phosphatase, serum alkaline phosphatase, cytochrome P-450

Hornig described that man who received no ascorbic acid (AsA) showed the four phases of AsA deficiency: first, preliminary deficiency state; next, biochemical deficiency state; then physiological deficiency state; and finally, clinically manifested deficiency state. The first three statuses can be understood as marginal vitamin C

¹ 鈴木恵美子, ² 倉田忠男, ¹ 荒川信彦

deficiency. In the biochemical deficiency state, no clinical symptoms of scurvy can be observed, but the reduction of AsA concentrations in plasma and leucocyte occur and the correlation between AsA levels and the activities of specific enzymes such as alkaline phosphatase can be noted (1).

Hornig *et al.* reported that administration of erythorbic acid (ErA), which is one of the stereoisomers of AsA, reduced AsA content in the tissues. The AsA content in the liver of the guinea pigs administered 5 mg AsA was about 80% higher than that of the guinea pigs administered simultaneously 5 mg of AsA and 50 mg of ErA (2). However, no information on the correlation between the reduction of AsA tissue level caused by ErA administration and the activities of the enzymes which depended on AsA level was reported. So, it is important to know whether ErA administration effects change in the activities of enzymes in guinea pigs as the administration of ErA reduced AsA content in the tissues of guinea pigs (9).

It is well known that the activities of liver drug metabolic enzymes, the content of liver cytochrome P-450, and the activity of serum alkaline phosphatase in AsA-deficient guinea pigs were significantly lower than those in AsA-supplemented guinea pigs, and that the activity of liver lysosomal enzymes in AsA-deficient guinea pigs was higher than that of normal animals (3-8).

In our previous paper, the activities of liver aniline hydroxylase, liver acid phosphatase, and serum alkaline phosphatase, and the content of liver cytochrome P-450 of the guinea pigs administered AsA together with various levels of ErA were measured to investigate the effect of ErA administration on the AsA availability (10). In this paper, the activities of the enzymes described above and the content of cytochrome P-450 in the guinea pigs administered 5 mg of AsA and/or 100 mg of ErA were also measured at varying intervals during the experimental period to clarify whether the administration of a large amount of ErA causes the biochemical changes or not.

MATERIALS AND METHODS

Animals and Diet. Male albino guinea pigs (Hartley strain) with initial body weights of about 220 g were used in the following experiments. All animals were housed individually in wire cages, and maintained on the AsA-deficient diet as described previously (9). Water and diets were offered *ad libitum*.

The AsA requirement of guinea pigs has been investigated (11). For instance, Collins and Elvehjem reported that 5 mg of AsA/kg body weight/day was sufficient for growth of guinea pigs (12). Boyle *et al.* found that 5 mg of AsA/day was necessary for a normal growth rate of guinea pig dentine (13). On the other hand, a supplementation of 200 mg of AsA/kg diet of guinea pigs was recommended by the National Research Council, National Academy of Sciences (14). In this paper, 5 mg of AsA/day as the basis of supplementation was used throughout this experiment, since this amount was regarded adequate to maintain normal health of guinea pigs (11-14). Vitamin C activity of ErA has been generally believed to be

one-twentieth that of AsA; therefore, 100 mg of ErA, which is equivalent to 5 mg of AsA, was set as the basis of supplementation.

Experimental plans. Experiment I: The animals were divided into four groups. Group A (control animals) was orally supplemented with 5 mg of AsA/day, group B with 100 mg of ErA/day, group C with both 5 mg of AsA and 100 mg of ErA/day, and group D was supplemented with neither AsA nor ErA. AsA and/or ErA were dissolved in water immediately before use and administered orally. On days 4, 10, 16, and 30 of the experimental period (group D was fed only up to 16 days), selected animals were sacrificed after 24-h fasting. Blood was collected from the aorta abdominalis and livers were removed.

Experiment II: The animals which were fed the AsA-deficient diet for 16 days to deplete their body store of AsA were divided into three groups. Group E was orally supplemented with 5 mg of AsA/day, group F with 100 mg of ErA/day and group G with both 5 mg of AsA and 100 mg of ErA/day. On days 0, 1, 4, and 20 of the repletion period, the animals were sacrificed after 24-h fasting, and the liver was removed. Blood was obtained from the aorta abdominalis.

Enzyme assay. The activity of liver aniline hydroxylase was determined as described in our previous paper (10), measuring the amount of *p*-aminophenol produced from aniline by the method of Imai *et al.* (15). Liver acid phosphatase activity was assayed using *p*-nitrophenylphosphate as substrate according to Desai (16) and serum alkaline phosphatase was assayed by using Alkaline Phosphatase B-Test (Wako Pure Chemical Industries, Osaka, Japan) (17). Liver microsome cytochrome P-450 content was determined by the method of Omura and Sato (18), measuring the carbon monoxide difference spectra of dithionite-reduced microsomes.

Determination of protein. Protein was measured by the method of Lowry *et al.* (19), using bovine serum albumin as a standard.

Statistical tests. The statistical significance difference between the means of the two groups of values was determined by either a Student's *t*-test or a Cochran-Cox test, depending on whether variances were equal or different.

RESULTS

Table 1 shows the liver aniline hydroxylase activity of the guinea pigs administered AsA and/or ErA. The aniline hydroxylase activities of AsA-supplemented guinea pigs (group A) of days 10 and 16 were significantly higher than those of AsA-deficient guinea pigs (group D). On the other hand, there was no difference in activity among these AsA-supplemented guinea pigs, ErA-supplemented guinea pigs, and both AsA and ErA-supplemented guinea pigs (groups A, B, and C).

The liver cytochrome P-450 content of the guinea pigs administered AsA and/or ErA is presented in Table 2. The content of cytochrome P-450 did not seem to change throughout the experimental period in groups A, B, and C, except for the

Table 1. Effect of ErA administration on liver aniline hydroxylase activity of guinea pigs supplemented with AsA and/or ErA.

Days of experiment	A	B	C	D
4	0.375 ± 0.042*	0.399 ± 0.065	0.307 ± 0.045	0.366 ± 0.041
10	0.254 ± 0.029 ^a	0.231 ± 0.051	0.201 ± 0.042	0.180 ± 0.009 ^a
16	0.240 ± 0.014 ^b	0.246 ± 0.025 ^c	0.219 ± 0.025 ^d	0.109 ± 0.015 ^{b,c,d}
30	0.272 ± 0.029	0.277 ± 0.010	0.284 ± 0.024	—

A, 5 mg AsA-supplemented group; B, 100 mg ErA-supplemented group; C, 5 mg AsA + 100 mg ErA-supplemented group; D, AsA-deficient group. * Values are means ± SE (nmol *p*-aminophenol formed/min/mg prot), *n* = 4–8 (except day 16 of group A, *n* = 13, day 16 of group D, *n* = 14). Means in the same horizontal row with a common superscript letter are significantly different. ^a *p* < 0.05; ^d *p* < 0.01; ^{b,c} *p* < 0.001.

Table 2. Effect of ErA administration on liver cytochrome P-450 content of guinea pigs supplemented with AsA and/or ErA.

Days of experiment	A	B	C	D
4	0.93 ± 0.07*	0.90 ± 0.04	1.05 ± 0.06	0.99 ± 0.10
10	0.88 ± 0.08	0.75 ± 0.06	0.91 ± 0.05	0.77 ± 0.04
16	0.86 ± 0.04 ^b	0.83 ± 0.06 ^c	0.87 ± 0.10 ^a	0.50 ± 0.05 ^{a,b,c}
30	0.99 ± 0.10	1.00 ± 0.14	1.03 ± 0.07	—

A–D, see Table 1 legend. * Values are means ± SE (nmol/mg prot), *n* = 5–8 (except day 16 of group A, *n* = 13, day 16 of group D, *n* = 14). Means in the same horizontal row with a common superscript letter are significantly different. ^a *p* < 0.01; ^{b,c} *p* < 0.001.

16-day AsA-deficient guinea pigs, which showed significant decrease in the content of cytochrome P-450, and also showed decrease in body weight (9).

Table 3 shows the effect of ErA administration on the activity of liver acid phosphatase in guinea pigs. The activity of acid phosphatase of the AsA-supplemented guinea pigs remained almost constant throughout the experimental period. But, the acid phosphatase activity of the 16-day AsA-deficient guinea pigs (group D) was significantly higher than that of the 16-day AsA-supplemented guinea pigs. On the other hand, this enzyme activity of the AsA-supplemented guinea pigs was not different from that of ErA-supplemented guinea pigs and that of both AsA and ErA-supplemented guinea pigs (groups B and C).

Table 4 shows the activity of serum alkaline phosphatase of the guinea pigs administered AsA and/or ErA. In the AsA-deficient guinea pigs (group D), this activity tended to decrease with the depletion of AsA. In the 10-day AsA-deficient guinea pigs, this activity seemed to be lower than that of AsA-supplemented guinea

Table 3. Effect of ErA administration on liver acid phosphatase activity of guinea pigs supplemented with AsA and/or ErA.

Days of experiment	A	B	C	D
4	45.3 ± 3.2*	48.2 ± 3.7	49.9 ± 4.5	50.6 ± 3.2
10	45.9 ± 3.5	47.5 ± 4.0	44.6 ± 1.2	45.3 ± 3.6
16	42.1 ± 2.3 ^c	44.9 ± 3.8 ^a	44.6 ± 1.4 ^b	58.9 ± 3.2 ^{a,b,c}
30	48.2 ± 2.5	51.8 ± 2.2	53.3 ± 1.5	—

A–D, see Table 1 legend. * Values are means ± SE (nmol *p*-nitrophenol liberated/min/mg prot), *n* = 5–8 (except day 16 of group A, *n* = 13, day 16 of group D, *n* = 13). Means in the same horizontal row with common superscript letter are significantly different. ^a *p* < 0.05; ^b *p* < 0.01; ^c *p* < 0.001.

Table 4. Effect of ErA administration on serum alkaline phosphatase activity of guinea pigs supplemented with AsA and/or ErA.

Days of experiment	A	B	C	D
4	5.47 ± 0.65*	4.45 ± 0.49 ^a	3.96 ± 0.40 ^b	5.28 ± 0.43 ^{a,b}
10	4.96 ± 1.20	5.07 ± 0.69	5.19 ± 0.79	3.81 ± 1.12
16	5.70 ± 0.49 ^d	5.04 ± 0.22 ^c	5.04 ± 0.56 ^c	1.21 ± 0.09 ^{c,d,e}
30	5.67 ± 0.50	4.90 ± 1.09	5.68 ± 0.40	—

A–D, see Table 1 legend. * Values are means ± SE (nmol *p*-nitrophenol liberated/h/liter), *n* = 3–6 (except day 16 of group A, *n* = 11, day 16 of group D, *n* = 12). Means in the same horizontal row with a common superscript letter are significantly different. ^{a,b} *p* < 0.05; ^c *p* < 0.01; ^{d,e} *p* < 0.001.

pigs but they did not show a decrease in body weights as reported in our previous paper (9). At 16 days, this activity of group D was significantly lower than that of groups A, B, and C. On the other hand, there was no significant difference in the activity among groups A, B, and C after 10 days of the experimental period.

Tables 5, 6, 7, and 8 show the activity of liver aniline hydroxylase, the content of liver cytochrome P-450, the activity of liver acid phosphatase, and the activity of serum alkaline phosphatase, respectively, of guinea pigs during the repletion period.

In the activity of liver aniline hydroxylase, all groups administered AsA and/or ErA showed a similar pattern of recovery. This activity at 0 day was lowest, but increased with the administration of AsA and/or ErA (groups E, F, and G). There was no statistically significant difference in the aniline hydroxylase activity among these three groups during the repletion period, except that at 20 days, the activity of group F was significantly lower than that of group E.

The pattern of recovery in liver cytochrome P-450 content of the guinea pigs

Table 5. Effect of ErA administration on liver aniline hydroxylase activity of guinea pigs supplemented with AsA and/or ErA during repletion period.

Days of repletion	E	F	G
0	0.109 ± 0.015 ^{a,b,c,d}		
1	0.129 ± 0.020	0.103 ± 0.026	0.122 ± 0.022
4	0.169 ± 0.020 ^{a,e}	0.173 ± 0.016 ^{c,f,g}	0.171 ± 0.027 ^h
20	0.239 ± 0.011 ^{d,e,f,h,i}	0.165 ± 0.026 ⁱ	0.192 ± 0.029 ^{b,g}

E, 5 mg AsA-supplemented group; F, 100 mg ErA-supplemented group; G, 5 mg AsA + 100 mg ErA-supplemented group. * Values are means ± SE (nmol *p*-aminophenol formed/min/mg prot), *n* = 4–7 (except day 0, *n* = 14). Means with a common superscript letter are significantly different. ^{a,b,e,h,i} *p* < 0.05; ^{c,f,g} *p* < 0.01; ^d *p* < 0.001.

Table 6. Effect of ErA administration on liver cytochrome P-450 content of guinea pigs supplemented with AsA and/or ErA during repletion period.

Days of repletion	E	F	G
0	0.50 ± 0.05 ^{a,b,c,d,e}		
1	0.51 ± 0.11	0.70 ± 0.15	0.69 ± 0.11
4	0.67 ± 0.04 ^{a,f}	0.70 ± 0.06 ^b	0.67 ± 0.10
20	0.96 ± 0.10 ^{c,f}	0.76 ± 0.08 ^c	0.78 ± 0.10 ^d

E–G, see Table 5 legend. * Values are means ± SE (nmol/mg prot), *n* = 4–9 (except day 0, *n* = 14). Means with a common superscript letter are significantly different. ^{a,b,c,d,f} *p* < 0.05; ^e *p* < 0.001.

Table 7. Effect of ErA administration on liver acid phosphatase activity of guinea pigs supplemented with AsA and/or ErA during repletion period.

Days of repletion	E	F	G
0	58.9 ± 3.2 ^{a,b,c}		
1	58.0 ± 3.5	58.2 ± 6.0	64.2 ± 3.4
4	59.1 ± 4.4 ^{d,e}	55.5 ± 4.7 ^{f,g}	64.0 ± 12.3
20	46.0 ± 4.9 ^a	42.2 ± 2.6 ^{b,d,f}	41.6 ± 3.3 ^{c,e,g}

E–G, see Table 5 legend. * Values are means ± SE (nmol *p*-nitrophenol liberated/min/mg prot), *n* = 4–9 (except day 0, *n* = 13). Means with a common superscript letter are significantly different. ^{a,f,g} *p* < 0.05; ^{b,c,d,e} *p* < 0.01.

administered AsA and/or ErA showed a tendency similar to that of aniline hydroxylase activity. The cytochrome P-450 content increased with administration of AsA and/or ErA, and the content of cytochrome P-450 of AsA-supplemented guinea pigs was not significantly different from that of ErA-supplemented guinea

Table 8. Effect of ErA administration on serum alkaline phosphatase activity of guinea pigs supplemented with AsA and/or ErA during repletion period.

Days of repletion	E	F	G
0	1.21 ± 0.09*		
1	1.63 ± 0.25	2.39 ± 0.40 ^a	1.99 ± 0.16 ^c
4	8.49 ± 0.70 ^c	9.33 ± 1.09 ^b	9.69 ± 1.35 ^b
20	6.10 ± 0.35 ^c	6.16 ± 0.65 ^b	6.28 ± 0.96 ^a

E–G, see Table 5 legend. * Values are means ± SE (nmol *p*-nitrophenol liberated/h/liter), *n* = 4–9 (except day 0, *n* = 12). Means with a superscript letter are significantly different from mean of day 0. ^a *p* < 0.05; ^b *p* < 0.01; ^c *p* < 0.001.

pigs and that of both AsA and ErA-supplemented ones during the repletion period.

The pattern of recovery in the liver acid phosphatase activity of AsA-supplemented guinea pigs was similar to that of ErA-supplemented guinea pigs and that of both AsA and ErA-supplemented ones. At 20 days of repletion, the activity of groups E, F, and G decreased to that of group A level. This activity of group E was not statistically different from those of groups F and G during the repletion period.

Serum alkaline phosphatase activity seemed to increase as soon as administration of AsA and/or ErA started. At 4 days of repletion, this enzyme activity of all three groups was level with that of group A, which was administered 5 mg of AsA throughout the experimental period. There was no difference in the enzyme activity among the three groups during the repletion period.

DISCUSSION

In experiment I, the activities of liver aniline hydroxylase and serum alkaline phosphatase, and the content of liver cytochrome P-450 of the AsA-deficient guinea pigs were significantly lower than those of the AsA-supplemented animals; the liver acid phosphatase activity was higher than in the AsA-supplemented guinea pigs at 16 days (Tables 1–4). The results obtained in this experiment agreed with the observations reported on scorbutic guinea pigs (3–8). These results indicate that a 16-day AsA deficiency could result in a biochemically scorbutic state in guinea pigs.

In the AsA-deficient guinea pigs, the activities of liver aniline hydroxylase and serum alkaline phosphatase, and the content of liver cytochrome P-450 seemed to be different from that of the AsA-supplemented guinea pigs after 10 days of the experimental period (Tables 1, 2, and 4). These activities seemed to change with decreasing content of AsA in the tissues.

Tables 5 to 7 show that until 4 days of repletion, the AsA-deficient guinea pigs showed slight recovery from a scorbutic state by administration of AsA and/or ErA, but completely recovered on 20 days. A difference in the recovery rate of the enzyme

activities was observed. For instance, the recovery rate of liver acid phosphatase activity was slow. On the other hand, at 1 day, only serum alkaline phosphatase activity was higher than that of 0 day, and at 4 days, this activity markedly increased in all three groups. As soon as the repletion started, this enzyme activity in these guinea pigs had been activated quickly. Serum alkaline phosphatase activity was considered to be sensitive to the change of AsA tissue levels.

The activities of enzymes which were measured in this study and the content of liver cytochrome P-450 of the guinea pigs administered both AsA and ErA were almost the same as those of the guinea pigs administered only AsA, as shown in Tables 1-8. But the liver AsA content of the guinea pigs administered both AsA and ErA was about 50% lower than that of the animals administered only AsA, as shown in a previous paper (9). The administration of ErA together with AsA reduced the AsA content in tissues, but did not have any effect on the activities of drug metabolic enzyme and phosphatases, and content of liver cytochrome P-450. The availability of AsA in the guinea pigs might not be reduced by the ErA administration.

In the 16-day ErA-supplemented guinea pigs, the tissue AsA was almost depleted and the liver ErA content was much lower than the liver AsA content of the AsA-supplemented guinea pigs (9). The activities of enzymes and the content of liver cytochrome P-450 in ErA-supplemented guinea pigs were significantly different from those of the AsA-deficient guinea pigs at 16 days; however, their values were similar to those of the AsA-supplemented guinea pigs. This indicated that the ErA-supplemented guinea pigs hardly retained ErA in the tissues (9), though they were not in the biochemically scorbutic state. Also, during the repletion period, the enzyme activities of the ErA-supplemented guinea pigs were similar to those of the AsA-supplemented guinea pigs. The administration of a large amount of ErA apparently prevented scurvy in guinea pigs, and produced almost a complete recovery from scurvy. The administration of ErA could keep the activity of the enzyme, which depended on the AsA tissue content, at normal levels.

Our results indicated that ErA may not affect the AsA availability in the guinea pigs, and may act as vitamin C if ErA was present in the tissues, though the mechanisms of absorption and storage of ErA seem to be considerably different from those of AsA.

REFERENCES

- 1) Hornig, D. (1982): Impact of marginal vitamin C deficiency. *J. Jap. Soc. Clin. Nutr.*, **3**, 127-141.
- 2) Hornig, D., and Weiser, H. (1976): Interaction of erythorbic acid with ascorbic acid catabolism. *Int. J. Vitam. Nutr. Res.*, **46**, 40-47.
- 3) Zannoni, V. G., Flynn, E. J., and Lynch, M. (1972): Ascorbic acid and drug metabolism. *Biochem. Pharmacol.*, **21**, 1377-1392.
- 4) Zannoni, V. G., and Sato, P. H. (1975): Effects of ascorbic acid on microsomal drug metabolism. *Ann. N. Y. Acad. Sci.*, **258**, 119-131.

- 5) Turnbull, J. D., and Omaye, S. T. (1980): Synthesis on cytochrome P-450 hem in ascorbic acid-deficient guinea pigs. *Biochem. Pharmacol.*, **29**, 1255–1260.
- 6) Omaye, S. T., and Turnbull, J. D. (1980): Effect of ascorbic acid on hem metabolism in hepatic microsomes. *Life Sci.*, **27**, 441–449.
- 7) Perkins, H. R., and Zilva, S. S. (1950): The influence of scurvy and fall in weight in young guinea pigs on the alkaline phosphatase content of the serum and the zones of provisional calcification. *Biochem. J.*, **47**, 306–318.
- 8) Hoehn, S. K., and Kanfer, J. N. (1978): L-Ascorbic acid and lysosomal acid hydrolase activities of guinea pig liver and brain. *Can. J. Biochem.*, **56**, 352–356.
- 9) Arakawa, N., Suzuki, E., Kurata, T., Otsuka, M., and Inagaki, C. (1986): Effect of erythorbic acid administration on ascorbic acid content in guinea pig tissues. *J. Nutr. Sci. Vitaminol.*, **32**, 171–181.
- 10) Suzuki, E., Kurata, T., Koda, M., and Arakawa, N. (1988): Effect of graded doses of erythorbic acid on activities of drug metabolic enzyme and phosphatases in guinea pigs. *J. Nutr. Sci. Vitaminol.*, **34**, 439–447.
- 11) Chatterjee, G. C. (1967): Ascorbic acid requirements of animals, in *The Vitamins*, Vol. 1, ed. by Sebrell, W. H., Jr., and Harris, R. B., Academic Press, New York, pp. 495–500.
- 12) Collins, M., and Elvehjem, C. A. (1958): Ascorbic acid requirement of the guinea pig using growth and tissue ascorbic acid concentrations as criteria. *J. Nutr.*, **64**, 503–511.
- 13) Boyle, P. E., Bessey, O. A., and Howe, P. R. (1940): Rate of dentin formation in incisor teeth of guinea pigs on normal and on ascorbic acid-deficient diets. *A. M. A. Arch. Pathol.*, **30**, 90–107.
- 14) National Academy of Sciences. National Research Council, U.S.A. (1978): *Nutrient Requirements of Domestic Animals*. No. 10, *Nutrient Requirements of Laboratory Animals*, Third revised edition, pp. 59–69.
- 15) Imai, Y., Ito, A., and Sato, R. (1966): Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J. Biochem.*, **60**, 417–428.
- 16) Desai, I. D. (1969): Regulation of lysosomal enzymes. I. Adaptive changes in enzyme activities during starvation and refeeding. *Can. J. Biochem.*, **47**, 785–790.
- 17) Bessey, O. A., Lowry, O. H., and Brock, M. J. (1946): A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.*, **164**, 321–329.
- 18) Omura, T., and Sato, R. (1964): The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.*, **239**, 2370–2378.
- 19) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.