RENIN RELEASE AND LIPID PEROXIDATION BY ASCORBIC ACID IN THE RENIN GRANULE FRACTION OF RAT KIDNEY CORTEX

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Abstract—This study was carried out to investigate the effect of lipid peroxidation in the renin granule fraction on renin release from the granules. Ascorbic acid was used to cause lipid peroxidation in the renin granule fraction prepared from rat kidney cortex homogenate. Renin activity was measured by radioimmunoassay and lipid peroxidation was estimated by means of the thiobarbituric acid test. Ascorbic acid, at the concentrations from 5 to 100 μM, produced a dose-dependent increase in lipid peroxidation during incubation of the renin granule fraction at 37°C for 30 min, accompanied by increased release of renin from the granules. On the other hand, dehydroascorbic acid showed no effects on lipid peroxidation and renin release. The simultaneous increases in lipid peroxidation and renin release induced by ascorbic acid in the renin granule fraction were markedly suppressed by the addition of disodium ethylenediaminetetra-acetic acid and antioxidants such as N,N'-diphenyl-p-phenylenediamine and hydroquinone. These findings indicate that lipid peroxidation in the renin granule fraction results in the stimulation of renin release from the granules.

There is general agreement that renin is stored in the granules of the juxtaglomerular cells. Previously, the release of renin from isolated granules has been investigated in response to changes in temperature, osmolality, pH and ionic composition of the incubation medium (1-4). Results indicated that renin granules were stable at 37°C without mechanical or osmotic shock and that stored renin in the granules was comprised of three components: a readily released soluble form, a soluble but hard-to-release form, and a membrane-bound form.

In recent years, lipid peroxidation has been considered to be an important factor which causes the impairment of membrane integrity. Several investigations have shown the close relationship between lipid peroxidation and increased permeability in red blood cells (5-7) and in subcellular particulates such as mitochondria (8-10) and lysosomes (11-13). However, there is little data available on lipid peroxidation in the renin granules.

The present study was designed, therefore, to investigate the effect of lipid peroxidation in the renin granule fraction on renin release from the granules.

Materials and Methods

Preparation of renin granule fraction: Male Wistar rats weighing 160-180 g were used. For at least 1 week before the study, the rats were fed a standard laboratory rat chow (Oriental Yeast Co., MF) and provided with tap water ad libitum.

After the peritoneal cavity was opened under pentobarbital sodium anesthesia (35 mg/kg, i.p.), both kidneys were removed and immediately cooled. The cortex was removed from the medulla, sectioned into thin slices.
and homogenized with ice-cold 0.45 M sucrose (1:8, wt./vol.). The renin granule fraction was prepared from the homogenate by a discontinuous sucrose density gradient centrifugation as previously described by us (14, 15). The homogenate was centrifuged at 500 g for 10 min to remove cell debris and nuclei. The supernatant was layered on a discontinuous sucrose density gradient solution (1.2 M to 1.7 M with 0.1 M intervals) and centrifuged at 60,000 g for 90 min. Renin granules were mainly equilibrated in the fraction corresponding to 1.5 M sucrose. This fraction was used as the renin granule fraction in the following experiment.

Preparation of microsomes and mitochondria: The renin granule fraction used in this study is contaminated by other subcellular organella such as microsomes and mitochondria, so we examined the effects of these subcellular particulates on lipid peroxidation and renin release in the renin granule fraction. Microsomes were prepared from the kidney cortex homogenate according to the procedure described previously (14, 16). As it is difficult to separate kidney mitochondria from renin granules, liver mitochondria were prepared from the liver homogenate by the method of Hogeboom (17). Microsomal and mitochondrial pellets were suspended in 0.1 M Tris-HCl buffer (pH 7.0) containing 0.15 M KCl (1.5 mg protein/ml).

Incubation system of renin granule fraction: Half milliliter of the renin granule fraction (0.45–0.50 mg protein) was suspended in 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.0) containing 0.15 M KCl. Ascorbic acid, dehydroascorbic acid, disodium ethylenediaminetetraacetic acid (EDTA), and antioxidants such as N,N'-diphenyl-p-phenylenediamine (DPPD) and hydroquinone were dissolved in the same buffer. These solutions were added to the renin granule fraction prior to the incubation. In the experiments using microsomes or mitochondria, 0.5 ml of the renin granule fraction was suspended in 1.0 ml of Tris-HCl buffer (pH 7.0) containing 0.15 M KCl and 0.2 mM ascorbic acid, and the mixture was made up to 2.0 ml with microsomal or mitochondrial suspension. In all experiments, the suspension was incubated at 37°C for 30 min, and then it was separated into the sediment and supernatant by centrifugation at 105,000 g for 60 min. The sediment was resuspended in Tris buffer (pH 7.0) and analyzed for renin activity, lipid peroxides and protein content. The supernatant was analyzed for renin activity.

Renin assay: Renin activity in the sample was measured by radioimmunoassay of angiotensin I (18) produced after incubation with semipurified rat renin substrate, which was prepared from the plasma of nephrectomized rats according to the procedure described previously (19). The incubation medium consisted of: a) samples containing renin, 0.05 ml; b) renin substrate dissolved in 0.25 M phosphate buffer (pH 7.0) containing 20 mM EDTA, 0.5 ml; c) 5% diisopropyl fluorophosphate, 20 μl; d) 6.6% 8-hydroxyquinoline sulfate, 10 μl; e) 10% dimercaprol, 3 μl. Incubation was carried out at 37°C for 15 min, and the reaction was stopped by heating for 5 min in a boiling water bath. Total renin was taken to be the sum of renin content in the sediment and supernatant, and the amount of renin release during incubation was expressed as a percentage of total renin.

Assay of lipid peroxides: Malondialdehyde (MDA) production, as measured by the thiobarbituric acid (TBA) test, served as an index of lipid peroxidation. MDA was measured according to the method of Ohkawa et al. (20). The reaction mixture consisted of: a) samples, 0.2 ml; b) 8.1% sodium dodecyl sulfate, 0.2 ml; c) 20% acetate buffer (pH 3.5), 1.5 ml; d) 0.8% TBA, 1.5 ml. The mixture was heated at 95°C for 60 min, and the reaction product was measured fluorometrically with excitation at 515 nm and emission at 553 nm.
after extraction with the mixture of n-butanol and pyridine (15:1, vol./vol.). Lipid peroxide formation was expressed in terms of MDA (nmol/mg protein) using tetramethoxypropane as the standard.

Measurement of protein contents: The protein contents were determined by the method of Lowry et al. (21) with the modifications that were described by Bensadoun and Weinstein (22) to eliminate the interference by Tris buffer.

Statistical analysis: Statistical analysis was performed using one-way analysis of variance for repeated measures combined with Dunnet’s multiple range tests (23).

Results

Effects of ascorbic acid on lipid peroxidation and renin release in the renin granule fraction: First, we determined the amounts of endogenous iron in the renin granule fraction by the o-phenanthroline method (24), and this indicated that this fraction contained 12.1±0.77 nmol iron per mg protein. Lipid peroxide formation in the renin granule fraction occurred slightly during incubation at 37°C for 30 min (1.80±0.30 nmol MDA/mg protein). When the renin granule fraction was incubated in the presence of 5 μM ascorbic acid, the lipid peroxidation was greatly stimulated, the lipid peroxide level being about 2.5 times the control. To evaluate the dose response, the concentrations of ascorbic acid added to the renin granule fraction were progressively increased from 5 to 100 μM. Compared with control incubations containing no ascorbic acid, each concentration of ascorbic acid increased the level of lipid peroxide formation during 30 min incubation. At a concentration of 100 μM, the level of lipid peroxides was approx. 7 times the control. On the other hand, when the renin granule fraction was incubated alone under the same conditions, 23.2±2.5% of total renin appeared in the medium. As shown in Fig. 1, renin release in the granule fraction increased progressively with increasing ascorbic acid concentrations in the incubation medium. At concentrations over 50 μM, the amount of renin release reached an extremely high value, i.e., 90–95% of total renin was released during incubation.

Effects of dehydroascorbic acid on lipid peroxidation and renin release in the renin granule fraction: The renin granule fraction was incubated with dehydroascorbic acid at different concentrations (10, 50 or 100 μM), but there were no significant changes in

Fig. 1. Effects of ascorbic acid on lipid peroxide formation and renin release in the renin granule fraction. Each column represents the mean of five separate experiments, and vertical bars indicate S.E. of the mean. *Values are significantly different from each control value (*P<0.01).

Fig. 2. Effects of dehydroascorbic acid on lipid peroxide formation and renin release in the renin granule fraction. Each point represents the mean of four separate experiments, and vertical bars indicate S.E. of the mean. —○—: lipid peroxide formation. —●—: renin release.
lipid peroxide formation and renin release (Fig. 2).

**Effects of EDTA on lipid peroxidation and renin release induced by ascorbic acid:**
When 10 μM EDTA was added to the renin granule fraction and then incubated in the presence of 50 μM ascorbic acid, the stimulation of lipid peroxidation by ascorbic acid was strongly inhibited to about 14% of the control level (to 1.85±0.29 from 13.30±0.10 nmol MDA/mg protein). Simultaneously, renin release in the renin granule fraction also decreased to the value of 29.8±2.9% from the control value of 96.9±2.3%. At a concentration of 100 μM, the inhibitory effects of EDTA were more potent, i.e., the level of lipid peroxide formation and the amount of renin release were 0.89±0.02 nmol MDA/mg protein and 15.5±1.4%, respectively (Fig. 3).

**Effects of antioxidants on lipid peroxidation and renin release induced by ascorbic acid:**
To further investigate the correlation between lipid peroxide formation and renin release, the following experiments were performed. Ascorbic acid and antioxidants such as DPPD and hydroquinone were simultaneously added to the renin granule fraction, and the suspension was incubated at 37°C for 30 min. As shown in Table 1, DPPD showed a concentration-dependent inhibition of lipid peroxide formation induced by ascorbic acid. Hydroquinone also produced a potent inhibitory action at concentrations over 100 μM. Simultaneously, the stimulation of renin release due to ascorbic acid was effectively suppressed by the addition of these antioxidants. At a concentration of 500 nM

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**Table 1. Effects of N,N'-diphenyl-p-phenylenediamine and hydroquinone on lipid peroxide formation and renin release induced by ascorbic acid**

| Drug          | Lipid peroxide formation (%) | Renin release (%) |
|---------------|-------------------------------|-------------------|
| No addition   | 100                           | 100               |
| DPPD (nM)     |                               |                   |
| 10            | 99.3±4.1                      | 101.5±0.3         |
| 50            | 61.8±9.5**                    | 65.2±6.5**        |
| 100           | 11.0±2.9**                    | 27.9±3.2**        |
| 500           | 5.7±0.9**                     | 25.7±3.1**        |
| Hydroquinone  |                               |                   |
| 10            | 93.5±5.3                      | 99.1±1.6          |
| 100           | 51.4±5.6**                    | 82.8±2.6*         |
| 1000          | 9.7±4.0**                     | 20.5±3.3**        |

The renin granule fraction (0.45–0.50 mg protein) was incubated with 50 μM ascorbic acid in the presence of indicated concentrations of antioxidants. Control incubations were performed in the absence of antioxidants. Control values for lipid peroxide formation and renin release were 12.5±1.8 nmol MDA/mg protein and 93.5±4.5%, respectively. Values are expressed as percentages of the control, and they are the means±S.E. of five separate experiments. *Values are significantly different from each control value (P<0.05, **P<0.01).
DPPD or 1 mM hydroquinone, the amount of renin release decreased below the level (23.2±2.5%) observed during incubation of the renin granule fraction alone as shown in Fig. 1. However, when 500 nM DPPD or 1 mM hydroquinone was added to the medium after preincubation of the renin granule fraction with 50 µM ascorbic acid at 37°C for 15 min, these antioxidants produced no effect on generated lipid peroxides and released renin.

Effects of the addition of microsomes or mitochondria on lipid peroxidation and renin release in the renin granule fraction: The amounts of endogenous iron of microsomes and mitochondria used were 10.9±0.78 and 11.1±0.57 nmol per mg protein, respectively. First, we examined the relation of protein concentration to lipid peroxide formation during incubation of microsomal or mitochondrial suspension with ascorbic acid. As shown in Fig. 4, a dilute suspension of microsomes or mitochondria (0.05-0.10 mg protein/ml) rapidly formed lipid peroxides, and total lipid peroxide formation showed a plateau above 0.2 mg protein/ml. Accordingly, the specific rate of lipid peroxide formation decreased with increasing microsomal or mitochondrial protein concentration. Next, the renin granule fraction was incubated with various concentrations of microsomal suspension. The specific rate of lipid peroxide formation in the incubation mixture decreased with increasing microsomal protein concentration, although total lipid peroxide formation was not changed by the addition of microsomes. The decrease in specific rate of lipid peroxide formation may be explained as increasing the substrate concentration for the peroxidative reaction. Simultaneously,

![Graph showing protein concentration and rate of lipid peroxide formation](image)

**Fig. 4.** Protein concentrations and the rate of lipid peroxide formation during incubation of microsomes or mitochondria with ascorbic acid. Various concentrations of microsomal or mitochondrial suspensions were incubated in the presence of 100 µM ascorbic acid. Each point represents the mean of four separate experiments, and vertical bars indicate S.E. of the mean.

| Added microsomes (mg protein) | Lipid peroxide formation (nmol MDA/ml)† | Renin release (nmol MDA/mg protein)§ | Renin release (%) |
|-------------------------------|----------------------------------------|--------------------------------------|------------------|
| 0                             | 2.23±0.32                              | 15.92±1.80                           | 94.6±2.13        |
| 0.15                          | 2.50±0.25                              | 12.86±0.57*                          | 85.4±2.37*       |
| 0.30                          | 2.42±0.24                              | 10.20±1.11**                         | 75.6±2.65**      |
| 0.45                          | 2.38±0.40                              | 7.75±0.30**                          | 61.7±4.54**      |
| 0.60                          | 2.39±0.28                              | 6.08±0.65**                          | 57.0±7.66**      |
| 0.75                          | 2.41±0.30                              | 6.03±0.84**                          | 50.1±5.58**      |

Indicated amounts of microsomes were added to the renin granule fraction (0.45-0.50 mg protein). The suspension was incubated in the presence of 100 µM ascorbic acid. Control incubations were performed without the addition of microsomes. Values are the means±S.E. of four separate experiments. *Values are significantly different from each control value (*P<0.05, **P<0.01). †: Total lipid peroxide formation, §: Specific rate of lipid peroxide formation.
Table 3. Effects of the addition of mitochondria on lipid peroxidation and renin release in the renin granule fraction

| Added mitochondria (mg protein) | Lipid peroxide formation (nmol MDA/ml)† | Renin release (% | Lipid peroxide formation (nmol MDA/mg protein)§ |
|---------------------------------|----------------------------------------|-----------------|-----------------------------------------------|
| 0                               | 1.88±0.16                              | 12.64±1.41      | 91.3±1.03                                      |
| 0.15                            | 2.01±0.17                              | 8.70±0.14**     | 81.6±2.92*                                    |
| 0.30                            | 2.18±0.21                              | 7.74±0.80**     | 69.4±1.83**                                   |
| 0.45                            | 1.92±0.16                              | 5.92±0.92**     | 56.5±2.47**                                   |
| 0.60                            | 2.05±0.14                              | 5.68±0.72**     | 45.2±3.01**                                   |
| 0.75                            | 2.07±0.10                              | 4.92±0.67**     | 44.2±2.83**                                   |

Indicated amounts of mitochondria were added to the renin granule fraction (0.45–0.50 mg protein). The suspension was incubated in the presence of 100 μM ascorbic acid. Control incubations were performed without the addition of mitochondria. Values are the means±S.E. of four separate experiments. *Values are significantly different from each control value (*P<0.05, **P<0.01). †: Total lipid peroxide formation. §: Specific rate of lipid peroxide formation.

renin release due to ascorbic acid was also attenuated by the addition of microsomes in a dose-related manner (Table 2). Similar results were obtained with the addition of liver mitochondria to the renin granule fraction (Table 3).

Discussion

The present study demonstrated that the release of renin was markedly stimulated by lipid peroxidation in the renin granule fraction.

In this study, we utilized ascorbic acid for lipid peroxidation in the renin granule fraction. It is known that iron components, probably inorganic iron ions, are essential for the formation of lipid peroxides in the presence of ascorbic acid. The requirement for an iron ion appears to be specific, and no other metal ions can replace it (25). The role of ascorbic acid is thought to maintain iron ions in their reduced form (26). The stimulatory effect of ascorbic acid on renin release was suppressed during incubation with these agents. However, after incubation of the renin granule fraction with ascorbic acid, the addition of these agents caused no changes in lipid peroxides and renin release. Thus, it seems that ascorbic acid causes the simultaneous increases in keep iron ions reduced, showed no significant influences on lipid peroxide formation and renin release. In addition, EDTA showed a potent inhibitory effect on lipid peroxidation and renin release due to ascorbic acid. At a concentration of 100 μM EDTA, lipid peroxide formation and renin release due to ascorbic acid were suppressed below the levels observed during incubation of the renin granule fraction alone. It is assumed that ferrous ion is the obligatory participant in lipid peroxidation of the renin granule fraction and that ascorbic acid keeps iron reduced and makes it available for the peroxidative reaction, as proposed by Sharma (27) in lipid peroxidation of brain mitochondria.

It has been reported that DPPD and hydroquinone show an antioxidative effect on lipid peroxide formation in microsomes (28, 29) and mitochondria (29, 30). In this study, these agents markedly inhibited the lipid peroxidation due to ascorbic acid. Furthermore, the stimulatory effect of ascorbic acid on renin release was suppressed during incubation with these agents. However, after incubation of the renin granule fraction with ascorbic acid, the addition of these agents caused no changes in lipid peroxides and renin release. Thus, it seems that ascorbic acid causes the simultaneous increases in
lipid peroxidation and renin release in the renin granule fraction.

However, it is difficult to say that lipid peroxidation occurs in the membrane of renin granules since the renin granule fraction is contaminated by other subcellular organelles, i.e., specific activities of glucose-6-phosphatase and succinate dehydrogenase in this fraction were about 10% and 40% of those of microsomes and mitochondria, respectively. On the other hand, there is a possibility that renin release may be enhanced by peroxidized substances derived from contaminating subcellular organelles such as microsomes and mitochondria. So, we examined the effect of the addition of microsomes or mitochondria on lipid peroxidation and renin release in the renin granule fraction. If ascorbic acid-induced renin release is enhanced by products originating from the peroxidation of contaminating microsomes or mitochondria, renin release would not be decreased by the addition of these subcellular organella. Actually, the addition of microsomes or mitochondria caused a dose-related decrease in renin release, accompanied by a decrease in the specific rate of lipid peroxide formation in the incubation mixture. These findings suggest that ascorbic acid-induced renin release in the renin granule fraction would not be due to the lipid peroxides of microsomal and mitochondrial membranes. Further studies are required for determining the precise relation of lipid peroxidation in the membrane of renin granules to renin release.

References
1) Yamamoto, K., Okahara, T., Abe, Y., Ueda, J., Kishimoto, T. and Morimoto, S.: Effects of cyclic AMP and dibutyl cyclic AMP on renin release in vivo and in vitro. Japan. Circ. J. 37, 1271–1276 (1973)
2) Yamamoto, K., Iwao, H., Abe, Y. and Morimoto, S.: Effect of Ca on renin release in vitro and in vivo. Japan. Circ. J. 36, 1127–1131 (1974)
3) Funakawa, S., Higashio, T. and Yamamoto, K.: Renin release from renin granules in the dog. Clin. Sci. Mol. Med. 55, 11–14 (1978)
4) Mannisto, P.T. and Poisner, A.M.: Further studies on properties of renin granules isolated from rat kidney cortex. Acta Physiol. Scand. 112, 365–371 (1981)
5) Schulze, R.M. and Kappus, H.: Lysis of erythrocytes as a result of microsomal lipid peroxidation induced by CCl₄ or FeCl₂. Res. Commun. Chem. Pathol. Pharmacol. 27, 129–137 (1980)
6) Goldstein, B.O., Rozen, M.G. and Kunis, R.L.: Role of red cell membrane lipid peroxidation in hemolysis due to phenylhydrazine. Biochem. Pharmacol. 29, 1355–1359 (1980)
7) Ribarov, S.R. and Benov, L.C.: Relationship between the hemolytic action of heavy metals and lipid peroxidation. Biochim. Biophys. Acta 640, 721–726 (1981)
8) Hunter, F.E., Jr., Scott, A., Hoffstein, P.E., Gorra, F., Weinstein, J., Schneider, A., Schutz, B., Fink, J., Ford, L. and Smith, E.: Studies on the mechanism of ascorbate-induced swelling and lysis of isolated liver mitochondria. J. Biol. Chem. 239, 604–613 (1964)
9) McKnight, R.C., Hunter, F.E., Jr. and Oehlert, W.H.: Mitochondrial membrane ghosts produced by lipid peroxidation induced by ferrous ion. I Production and general morphology. J. Biol. Chem. 240, 3439–3446 (1965)
10) Shimada, O. and Yasuda, H.: Lipid peroxidation and its inhibition by tinoridine. II Ascorbic acid-induced lipid peroxidation of rat liver mitochondria. Biochim. Biophys. Acta 572, 531–536 (1979)
11) Desai, I.D., Sawant, P.L. and Tappel, A.L.: Peroxidation and radiation damage to isolated lysosomes. Biochim. Biophys. Acta 86, 277–285 (1964)
12) Wills, E.D. and Wilkinson, A.E.: Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. Biochem. J. 99, 657–666 (1966)
13) Goto, K., Hisadome, M. and Imamura, H.: Effects of tinoridine on stability of rat liver and kidney lysosomes, and liver parenchymal cells. Biochem. Pharmacol. 26, 11–18 (1977)
14) Morimoto, S., Yamamoto, K. and Ueda, J.: Isolation of renin granules from the dog kidney cortex. J. Appl. Physiol. 33, 306–311 (1972)
15) Morimoto, S., Abe, R., Fukuhara, A., Tanaka, K. and Yamamoto, K.: Effect of sodium restriction on plasma renin activity and renin granules in rat kidney. Am. J. Physiol. 237, F367–F371 (1979)
16) Morimoto, S., Tanaka, K. and Kitano, R.:
Distribution of renin in subcellular fractions from the rabbit kidney. Japan. J. Pharmacol. 25, 295–301 (1975)

17) Hogeboom, G.H.: Fractionation of cell components of animal tissues. Methods Enzymol., Edited by Colowick, S.P. and Kaplan, N.O., Vol. 1, p. 16–18, Academic Press, New York and London (1955)

18) Haber, E., Koerner, T., Page, L.B., Kliman, B. and Purnode, A.: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J. Clin. Endocrinol. Metab. 28, 1349–1355 (1969)

19) Morimoto, S., Yamamoto, K., Horiuchi, K., Tanaka, H. and Ueda, J.: A release of renin from dog kidney cortex slices. Japan. J. Pharmacol. 20, 536–545 (1970)

20) Ohkawa, H., Ohishi, N. and Yagi, K.: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358 (1979)

21) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

22) Bensadoun, A. and Weinstein, D.: Assay of proteins in the presence of interfering materials. Anal. Biochem. 70, 241–250 (1976)

23) Dunnet, C.W.: A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121 (1955)

24) Sandell, E.B.: Colorimetric Determination of Traces of Metals, 2nd ed., p. 375–378, Inter- science Publishers, Inc., New York (1950)

25) Wills, E.D.: Lipid peroxide formation in microsomes: The role of non-haem iron. Biochem. J. 113, 332–332 (1969)

26) Ottolenghi, A.: Interaction of ascorbic acid and mitochondrial lipids. Arch. Biochem. Biophys. 79, 355–365 (1959)

27) Sharma, O.P.: Ascorbic acid, iron and non-enzymic lipid peroxidation in rat brain mitochondria. Indian J. Biochem. Biophys. 16, 139–142 (1979)

28) Slater, T.F. and Sawyer, B.C.: The stimulatory effects of carbon tetrachloride on peroxidative reactions in rat liver fractions in vitro: Inhibitory effect of free-radical scavengers and other agents. Biochem. J. 123, 823–828 (1971)

29) Inouye, B., Morita, K., Ishida, T. and Ogata, M.: Cooperative effect of sulfite and vanadium compounds on lipid peroxidation. Toxicol. Appl. Pharmacol. 53, 101–107 (1980)

30) Fujita, T. and Fujimoto, Y.: Effect of various diuretics on lipid peroxidation in rat renal cortical mitochondria and in the supernatant. Japan. J. Pharmacol. 31, 795–800 (1981)