Biochemical Pharmacology, Vol. 32, No. 20, pp. 3063–3067, 1983.
Printed in Great Britain.

0006–2952/83 $3.00 + 0.00

SELENIUM AND DRUG METABOLISM—I

MULTIPLE MODULATIONS OF MOUSE LIVER ENZYMES

RUDOLF REITER and ALBRECHT WENDEL*
Physiologisch-Chemisches Institut der Universität, Hoppe-Seyler Strasse 1, D-7400 Tübingen, Federal Republic of Germany

(*Received* 23 *February* 1983; *accepted* 2 *May* 1983)

Abstract—Male albino mice were raised on diets containing less than 10 ppb selenium (Se−) or supplemented with 0.5 ppm selenium (Se+) for 6 months. In the (Se−) group total liver selenium was less than 10% of the control, liver selenium-dependent glutathione peroxidase (GSH-Px) less than 2%. The specific activities of catalase and superoxide dismutase showed essentially no differences between the dietary groups. Several phase I-related specific enzyme activities were measured in liver microsomes. No significant differences between the two animal groups were found for cytochrome P-450 and *b* 5 content, NADH-cytochrome *b* 5 reductase, as well as for aniline hydroxylation and aminopyrine dealkylation rates. In (Se−) microsomes, NADPH-cytochrome P-450 reductase activity was about half that found in (Se+) microsomes. An *increase* in microsomes from (Se−) mice was found for 7-ethoxycoumarine deethylation rate (460%), cytochrome P-450 hydroperoxidase activity (170%), and heme oxygenase (276%). The *N*-oxidation rate of the flavin-containing monooxygenase decreased by 35%, the *N*-demethylation rate by 50% in (Se−) animals.

Stopped-flow measurements of the reduction rates of microsomal pigments did not support evidence for limitations in microsomal electron supply during selenium deficiency. Among the phase II reactions examined, sulfotransferase activity towards 4-nitrophenol was 47% of the controls in Se-deficient liver cytosols while UDP-glucuronyl transferase activity towards this substrate increased to 215%. Glutathione-*S*-transferase activity was much higher in (Se−) livers than in (Se+): 310% with 1,2-dichloro-4-nitrobenzene, 255% with 1-chloro-2,4-dinitrobenzene and 120% with ethacrynic acid as substrate.

The data indicate that in addition to GSH-Px many other enzyme activities in mouse liver are affected by prolonged dietary selenium deficiency. These effects might be useful in assessing the severity of selenium deficiency. A microsomal selenium-dependent metabolic modulator is discussed as a possible mechanism.

Selenium, though known to be toxic for at least one century, was established as an essential biologically active trace element in 1957 [1, 2]. Till now, glutathione peroxidase (GSH-Px) remains the only mammalian selenoprotein which is clearly characterized with respect to stoichiometry [3] and catalytic function [4] of selenium in its active site [5]. The capability of this enzyme to reduce fatty acid hydroperoxides [6] led to the conclusion that the enzyme [7] and hence selenium [8] might be an important determinant in the protection of cellular structures against oxidative challenge. This view was corroborated by the observation that Se-deficient rodents were much more susceptible to some types of xenobiotic-induced liver necrosis than adquately nourished animals [9–11]. Burk *et al.*, however, noted that this might not reflect only the impaired function of GSH-Px [10]. Moreover, additional effects of selenium deficiency different from GSH-Px activity were reported, such as an impairment of heme metabolism [12], an enhanced GSH turnover in (Se−) rat liver hepatocytes [13], increased hepatic GSH-*S*-transferase activity in the livers of (Se−) rats [14] and mice [15], and alterations in the inducibility of microsomal hemoproteins in rats [16]. This prompted us to study the influence of long-term dietary selenium deficiency on the hepatic metabolism of foreign compounds *in vitro* in order to assess to what extent endogenous factors other than GSH-Px might influence drug metabolism and toxicity.

MATERIALS AND METHODS

Weanling male albino mice (strain NMRI han) were purchased from the Zentralinstitut für Versuchstiere, Hannover, F.R.G. and housed in plastic cages in groups of 6–8 animals. They were fed a Torula yeast-based diet formulated by and received from Dr. R. F. Burk, San Antonio, TX, which was adequate in vitamin E and sulfur amino acid requirements [17]. Its selenium content was less than 10 ppb selenium. A control diet of the same composition supplemented with 0.5 ppm selenium in the form of sodium selenite was given to control groups.

After 6 months the animals were fasted 1 day and killed by cervical dislocation. Blood was sampled from the vena cava and serum was separated by centrifugation. Livers were perfused with ice-cold saline, excised and homogenized in 0.1 mole/l. potassium phosphate buffer, pH 7.4, containing 0.1 mmole/l. ethylenediaminetetraacetate, 1 mmole GSH and 0.1% fatty acid-free bovine serum albumin. Microsomal and cytosolic fractions were isolated by differential centrifugation [18]. Microsomes were washed twice in 0.1 mole/l. potassium phosphate, pH 7.4, and used for the assays within 6 hr after

*Author to whom all correspondence should be addressed.

isolation. For selenium determination, whole livers were lyophilized, wet-ashed and selenium was determined as the piazselenol formed after reaction with diaminonaphthalene [19] using an Aminco JA-74 39 fluorometer. Serum vitamin E was extracted and measured by high performance liquid chromatography as described in [20]. Reduced *plus* oxidized glutathione (GSH + 2GSSG) was determined by the coupled test via the NADPH/GSSG-reductase system as reported previously [21]. The enzymatic activities of GSH-Px, superoxide dismutase, catalase and glutathione-*S*-transferases were determined essentially as described previously [11]. Cytochrome P-450 and *b* 5 contents were assayed according to [22]. NADPH-cytochrome P-450 reductase [23] and NADH-cytochrome *b* 5 reductase [24] were assayed in the presence of 0.1 μmole/l. rotenone. Monooxygenase activity towards aniline [25], aminopyrine [26] and ethoxycoumarine [27] was measured as described elsewhere. Microsomal hydroperoxidase activity was assayed using cumene hydroperoxide as substrate [28], and hydrogen peroxide production (i.e. cytochrome P-450 oxidase activity) was determined via the peroxidatic reaction of catalase in the presence of methanol [29]. Microsomal heme oxygenase activity was measured in coupled test as bilirubin formation [30, 31] using excess cytosol as a source of biliverdin reductase. Flavin-containing monooxygenase was assayed in 0.1 mole/l. Tris-(hydroxymethyl)-aminomethane of pH 8.4 in the presence of 3 mmole/l. octylamine, 1 mmole/l. NADPH and 1 mmole/l. *N,N*-dimethylaniline. *N*-Oxide formation and *N*-demethylation were measured according to [32]. Glucuronidation was assayed in the presence of 0.5% Tween 80 using *p*-nitrophenol as substrate [33]. This was also used for the determination of the cytosolic sulfotransferase activity [34]. Protein was determined according to Lowry *et al.* [35].

The stopped flow experiments were performed with the rapid mixing attachment RMA-1A mounted on a Shimadzu UV-300 dual wavelength spectrophotometer connected to a recording oscilloscope. Reduction of cytochrome P-450 was followed by the formation of the CO-complex [36] during anaerobiosis at the wavelength couple 450–490 nm. Cytochrome *b* 5 reduction was monitored under aerobic conditions as absorbance change at 423–409 nm. The autoxidation rate of cytochrome *b* 5 was determined according to [37].

All assays were run in duplicate at room temperature (22°) In contrast, GSH-Px, sulfotransferase and flavin-containing monooxygenase were measured at 37°. NADPH, NADH, GSH, GSSG-reductase, catalase and cytochrome *c* were from Boehringer (Mannheim, F.R.G.). 7-Ethoxycoumarine, umbelliferone, UDP glucuronic acid, *N,N*-dimethylaniline, ethacrynic acid, *p*-nitrophenol, heme, rotenone, bovine serum albumin, D,L,α-tocopherol acetate and superoxide dismutase were from Sigma (München, F.R.G.). *o*-Dianisidine, riboflavin, Tween 80 and D-α-tocopherol were from Serva (Heidelberg, F.R.G.). 1,2-Dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene as well as tetramethylphenylene diamine were from Fluka (Buchs, Switzerland). *t*-Butylhydroperoxide and cumene hydroperoxide were supplied by Peroxid-Chemie, (Höllriegelskreuth, F.R.G.). All other chemicals were *pro-analysi* substances from Merck (Darmstadt, F.R.G.)

RESULTS

Mice fed a selenium-deficient diet for 6 months from weanling gained about 15% less weight than selenium-supplemented control animals (Table 1). While serum vitamin E, liver protein content and the liver glutathione content were not significantly different between the two dietary groups, total liver selenium was 8% of the control in the deficient animals. The decrease in the activity of the seleno-enzyme GSH-Px was even much more pronounced (Table 2): using H_2O_2 as substrate as an exclusive test for the selenium-dependent mouse liver enzyme, a residual activity of 1.2% was found in the (Se−) animals. Similarly, with *t*-butylhydroperoxide, 3.3% activity was found, while with cumene hydroperoxide —which includes the non-selenium dependent activity combined with a higher maximum velocity [15]— a drop to about one quarter was observed in (Se−) mice. It is noted that this cumene hydroperoxide-reactive activity contains a part of GSH-*S*-transferases which increased in (Se−) livers (cf. Table 4). On the other hand, catalase as well as superoxide dismutase activities were not affected by this prolonged dietary selenium deficiency.

Table 3 is a compilation of the dependence of detailed enzymic parameters related to phase I reactions of microsomal drug metabolism on the selenium status of the animals. While NADH-cytochrome *b* 5 reductase activity remained unaffected by selenium deficiency, NADPH-cytochrome P-450 reductase activity assayed either with ferricyanide or with ferricytochrome *c* as electron acceptor showed about half the specific activity of the control livers. Further detailed insight into this phenomenon was possible by inspection of the individual rate

Table 1. Assessment of the dietary status of mice fed a torula yeast-based diet

	Se(−)	Se(+)
Body weight (g)	45.6 ± 3.15*	53 ± 4.1
Serum vitamin E (μg/ml)	4.0 ± 0.6	4.8 ± 1.0
Liver protein (mg/g wet wt)	164 ± 26	173 ± 30
Liver glutathione (nmole/mg)	18.2 ± 1.6	19.5 ± 1.5
Liver selenium (ng/g wet wt)	65 ± 21***	781 ± 60

Means ± S.D., $n = 6$.
* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

Table 2. Specific activities of mouse liver enzymes involved in reactive oxygen metabolism in selenium-deficient and selenium-supplemented mice

	Se(−)	Se(+)
Glutathione peroxidase (mU/mg)†		
Hydrogen peroxide as substrate	9.2 ± 7.8*	733.8 ± 93.6
t-Butyl hydroperoxide	27.4 ± 3.4*	816 ± 85
Cumene hydroperoxide	424.5 ± 61*	1176 ± 162
Catalase (mK/mg)‡	547.4 ± 120	530 ± 174
Superoxide dismutase (pmol/mg)‡	233 ± 29	220 ± 32

* $P < 0.0005$.
† Cytosol.
‡ Homogenate.

constants for the reduction of the microsomal pigments: the relevant fast phases of microsomal pigment reduction or autoxidation were essentially independent of dietary selenium even though the slow phase of the reduction of cytochrome P-450 by NADPH was sensitive to selenium deficiency, and its decrease in (Se−) microsomes was quantitatively similar to the decrease of the overall enzymatic rate. This lack of significant changes in the reduction kinetics of microsomal pigments suggests that in selenium deficiency no limitations of the microsomal electro flow occur at the reductase level.

Further data in Table 3 show, however, that the composition of cytochrome P-450 species may vary between (Se−) and (Se+) animals. On the one hand, no significant selenium-dependent changes were observed for the aniline hydroxylation, aminopyrine demethylation and the P-450 oxidase activities. On the other hand, the cumene hydroperoxide-supported P-450 hydroperoxidase activity was much higher in (Se−) microsomes than in controls, and a very large increase of the ethoxycoumarine deethylation rate was observed in those microsomes. Further data in Table 3 demonstrate that this modulation of microsomal enzyme activities does not seem to be confined to the cytochrome P-450 cycle itself but that also other reactions withdrawing electrons via NADPH-cytochrome P-450 reductase are

Table 3. Selenium-dependent alterations in mouse liver microsomal parameters involved in phase I drug metabolism

	Se(−)	Se(+)
CYTOCHROME CONTENT (nmole/mg)		
Cytochrome P-450	0.82 ± 0.07	0.85 ± 0.12
Cytochrome *b* 5	0.35 ± 0.05	0.36 ± 0.05
MICROSOMAL ENZYME ACTIVITIES (nmole/mg min)		
NADPH-cytochrome P-450 reductase		
Ferricyanide	322.5 ± 48.3**	638.3 ± 114.3
Cytochrome *c*	97.4 ± 17**	157.2 ± 23.2
NADH-cytochrome *b* 5 reductase		
Ferricyanide	4522 ± 231	5023 ± 194
Cytochrome *c*	667 ± 91	797 ± 108
Cytochrome P-450 monooxygenase		
Aniline	0.46 ± 0.07	0.49 ± 0.05
Aminopyrine	6.4 ± 0.7	6.25 ± 0.6
7-Ethoxycoumarine	6.2 ± 0.9**	1.3 ± 0.2
Cytochrome P-450 oxidase	5.3 ± 1.1	4.1 ± 0.3
Cytochrome P-450 hydroperoxidase	98 ± 22.5*	57 ± 10.5
Heme oxygenase	0.18 ± 0.03**	0.06 ± 0.02
Flavin-containing monooxygenase		
N-Oxide formation	2.6 ± 0.4*	4.0 ± 0.42
N-Demethylation	0.88 ± 0.1**	1.8 ± 0.3
RATE CONSTANTS: k (sec^{-1})		
Reduction of cytochrome P-450 by NADPH		
Fast phase	0.22 ± 0.04	0.24 ± 0.05
Slow phase (10^3k)	3.5 ± 0.32*	5.5 ± 0.35
Reduction of cytochrome *b* 5 by NADPH		
Fast phase	2.5 ± 0.3	2.5 ± 0.1
Slow phase	0.09 ± 0.01	0.08 ± 0.03
Reduction of cytochrome *b* 5 by NADH		
Fast phase	4.7 ± 0.09	4.6 ± 0.1
Slow phase (10^3k)	0.06 ± 0.01	0.07 ± 0.02
Autoxidation of cytochrome *b* 5 ($10\,k$)	11.8 ± 0.7	11.5 ± 0.8

* $P < 0.005$, ** $P < 0.0005$.

Table 4. Influence of prolonged dietary selenium deficiency on mouse liver conjugation reactions related to drug metabolism

	Se(−)	Se(+)
Glutathione-*S*-transferases (mU/mg)†		
1,2-Dichloro-4-nitrobenzene	80.5 ± 7.0***	26 ± 2.6
1-Chloro-2, 4-dinitrobenzene	7381 ± 810***	2884 ± 550
Ethacrynic acid	144.5 ± 16*	120 ± 16
UDP-Glucuronyl transferase (mU/mg)‡		
4-Nitrophenol	3.5 ± 0.7***	1.13 ± 0.3
Sulfotransferase (mU/mg)†		
4-Nitrophenol	0.18 ± 0.04**	0.37 ± 0.1

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.
† Cytosolic.
‡ Microsomal.

affected. Heme oxygenase activity was much higher in the liver microsomes of (Se−) mice. Moreover, *N*-oxidation as well as *N*-demethylation catalysed by the flavin-containing monooxygenase was much lower in these animals. This suggests that other enzymes which are supplied with reducing equivalents via the microsomal electron transport system are also modulated by dietary selenium deficiency with oppositely directed effects in this case. After this characterization of phase I reactions it seemed necessary to account also for possible influences of selenium on phase II reactions of mouse liver drug metabolism. Data in Table 4 show that in addition to the known increase in GSH-*S*-transferase activity in liver cytosol of (Se−) animals [15], glucuronyl transferase activity in (Se−) liver microsomes was very high compared to the controls while cytosolic sulfotransferase activity was drastically decreased. Again, these results stress the diversity of the modulatory action of selenium on drug metabolism.

DISCUSSION

A decrease in GSH-Px activity below detection limit has been widely used as a biological criterion to define selenium deficiency in animals and man [38]. Obviously, in the liver many enzymatic processes other than GSH-Px are affected by prolonged dietary selenium deficiency. Their heterogenous pattern cannot be accounted for by compartmentation or metabolic inter-relationships, e.g. phase I or phase II reactions of drug metabolism. By the effect evoked they may be subdivided into three classes: (1) a small number of unchanged enzymes; (2) the activity of a group of enzymes which is at least doubled in selenium deficiency, i.e. ethoxycoumarine deethylase, P-450 hydroperoxidase, GSH-*S*-transferases, and glucuronyl transferase activities; and (3) a class of enzymatic rates that drop to about one half or less of its normal (Se+) amount, i.e. NADPH-cytochrome P-450 reductase, flavin-dependent monooxygenase and sulfotransferase activity. Several of these modulations have been singly reported for selenium-deficient rats, such as increased glucuronyl transferase [39], glutathione-*S*-transferases [12] and microsomal hydroperoxidase [40] activity. On the other hand, the monooxygenase activity of rat liver microsomal cytochrome P-450 has been described as independent of [40], or decreased [41, 42] in, selenium deficiency. In spite of species differences, a general agreement among these reports and our data seem to prevail. Incidentally, some of the observed increases of enzymatic parameters provide a convenient and sensitive diagnostic tool to assess severe selenium deficiency.

The observed drastic changes in drug-metabolizing enzymes of selenium-deficient mouse liver need a rationale which hardly can be deduced at the present time. We consider three hypotheses on different levels of metabolic organization which are discussed in increasing order of their presumptive relevance:

(A) In a preliminary time course study we observed that these changes in drug metabolism occur in a very late phase of selenium depletion after liver GSH-Px activity had completely disappeared. Hence a general impairment of membrane function as a consequence of GSH-Px deficiency could lead to changes of drug-metabolizing enzymes. Clinical observations as well as the kinetic properties [43] of selenium-dependent GSH peroxidase suggest that low amounts of the enzyme may be sufficient for maintenance of metabolic competence, though high activities are required for metabolic risk management. Similar manifold alterations were observed in various vitamin E deficient species [40].

(B) Feeding the semi-synthetic diet used here possibly shortened the supply of another micronutritient related to enzymes examined in our study. Therefore, a latent deficiency of some micronutritient may have been aggravated by selenium deficiency and misinterpreted as such. The mutual sparing effects of vitamin E, sulfur amino acids and selenium [44] provide evidence for these inter-relationships. The latter has been taken into account by supplementing our diet with relatively high amounts of methionine and vitamin E [17]. However, also interactions of other essential trace elements, e.g. among selenium, copper and iron, are described [45]. Thus, hitherto unknown inter-relations of nutritional components cannot be excluded.

(C) The recent isolation of some functionally, as yet uncharacterized, mammalian selenoproteins [46–48] illustrates our lack of knowledge on biologically active forms of selenium, apart from GSH-Px. Burk *et al.* [16] calculated a

microsomal selenium content of 0.8 nmole per g rat liver, which is about the same concentration as several microsomal enzymes. From these and other data a factor involved in microsomal electron transport has been proposed ([49] and R. F. Burk, personal communication). Incidentally, we identified microsomal selenium-containing proteins of subunit molecular weights of 18 and 47 kilodaltons which incorporate selenium more avidly than GSH-Px (R. Reiter and A. Wendel, unpublished results). The involvement of a selenoprotein in either an enzymatic or a regulatory function seems conceivable.

The overall influence of selenium on xenobiotic metabolism is likely to become very complex, since routes of metabolic activation and pathways removing potentially reactive intermediates are positively or negatively affected and will lead to different product patterns for individual drug substrates. This means practically that either the role of GSH-Px as a protective mechanism will be overestimated or the metabolic activation of a drug will be underestimated, if toxicity of xenobiotics is examined in selenium-deficient animals. It should be especially considered whether the effects of selenium supplementation of mammals become predictable in the light of the results reported here. Actually, selenium deficiency exacerbated the hepatotoxicity of drugs such as paracetamol, furosemide, ethylmorphine and aminopyrine in mice, if administered together with the GSH-depletor diethylmaleate [11]. Using similar dietary conditions in rats less toxicity was found for iodipamide and paracetamol while the toxicity of the redox cyclers diquat and paraquat was greatly enhanced [50].

Acknowledgement—The fruitful exchange of information with Dr. R. F. Burk, the Texas Health Science Center, San Antonio, Texas, is gratefully acknowledged.

REFERENCES

1. K. Schwarz and C. M. Foltz, *J. Am. chem. Soc.* **79**, 3292 (1957).
2. T. A. Noguchi, H. Cantor and M. L. Scott, *J. Nutr.* **103**, 1502 (1957).
3. L. Flohe, W. A. Günzler and H. H. Schock, *FEBS Lett.* **32**, 132 (1973).
4. A. Wendel, W. Pilz, R. Ladenstein, G. Sawatzki and U. Weser, *Biochim. biophys. Acta* **377**, 211 (1975).
5. R. Ladenstein, O. Epp, K. Bartels, A. Jones, R. Huber and A. Wendel, *J. molec. Biol.* **134**, 199 (1979).
6. B. O. Christophersen, *Biochim. biophys. Acta* **164**, 35 (1968).
7. L. Flohé, *Klin. Wochenschr.* **49**, 669 (1970).
8. W. G. Hoekstra, *Fedn. Proc.* **34**, 2083 (1975).
9. D. G. Hafeman and W. G. Hoekstra, *J. Nutr.* **107**, 666 (1977).
10. R. F. Burk, R. A. Lawrence and J. M. Lane, *J. clin. Invest.* **65**, 1024 (1980).
11. A. Wendel and S. Feuerstein, *Biochem. Pharmac.* **30**, 2513 (1981).
12. R. F. Burk, R. A. Lawrence, and M. A. Correia, *Biochem. Pharmac.* **29**, 39 (1980).
13. K. E. Hill and R. F. Burk, *J. biol. Chem.* **257**, 10668 (1982).
14. R. A. Lawrence, L. K. Parkhill and R. F. Burk, *J. Nutr.* **108**, 981 (1978).
15. P. Cikryt, S. Feuerstein and A. Wendel, *Biochem. Pharmac.* **31**, 2873 (1982).
16. R. F. Burk, A. M. Mackinnon and F. R. Simon, *Biochem. biophys. Res. Commun.* **56**, 431 (1974).
17. R. A. Lawrence and R. F. Burk, *J. Nutr.* **108**, 211 (1978).
18. C. Mitoma, H. S. Potter, H. C. Reitz and S. Udenfriend, *Archs. Biochem. Biophys.* **61**, 431 (1956).
19. J. H. Watkinson, *Analyt. Chem.* **38**, 92 (1966).
20. J. G. Bieri, T. J. Tolliver and G. L. Catignani, *Am. J. clin. Nutr.* **32**, 2143 (1979).
21. A. Wendel, S. Feuerstein and K. H. Konz. *Biochem. Pharmac.* **30**, 2051 (1979).
22. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
23. H. W. Strobel and J. D. Digman, *Methods Enzym.* **52**, 97 (1978).
24. K. Mikara and R. Sato, *Methods Enzym.* **52**, 102 (1978).
25. Y. Imai, A. Ho and R. Sato, *J. Biochem.* **60**, 412 (1966).
26. J. Werringloer, *Methods Enzym.* **52**, 297 (1978).
27. V. U. Ullrich and B. Weber, *Hoppe-Seyler's. Z. physiol. Chem.* **353**, 1171 (1972).
28. P. J. O'Brien and A. D. Rahimtula, *Methods Enzym.* **52**, 407 (1978).
29. A. G. Hildebrandt, I. Roots, M. Tjoe and G. Heinemeyer, *Methods Enzym.* **52**, 342 (1978).
30. R. Tenhunen, H. S. Marver and R. Schmid, *J. biol. Chem.* **244**, 6388 (1969).
31. M. A. Correia and R. Schmid, *Biochem. biophys. Res. Commun.* **65**, 1378 (1975).
32. D. M. Ziegler and F. H. Pettit, *Biochem. biophys. Res. Commun.* **15**, 188 (1964).
33. J. P. Gorski and C. G. Kasper, *J. biol. Chem.* **252**, 1336 (1977).
34. R. S. Yang and C. F. Wilkinson, *Biochem. J.* **130**, 487 (1972).
35. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
36. T. Matsubara, J. Baron, L. L. Peterson and J. A. Peterson, *Archs. Biochem. Biophys.* **172**, 463 (1976)
37. N. Oshino and R. Sato, *J. Biochem.* **69**, 169 (1971).
38. H. E. Ganther, D. G. Hafeman, R. A. Lawrence, R. E. Serfass and W. G. Hoekstra, in *Trace Elements in Human Health and Disease* (Eds. A. S. Prasad and D. Oberleas), Vol. 2, p. 165. Academic Press, New York (1976).
39. J. Chen, M. P. Goethius, T. Champell and G. F. Combs, Jr., *J. Nutr.* **112**, 324 (1982).
40. G. L. Catignani, in *Vitamin E, a Comprehensive Treatise* (Ed. J. E. Machlin), p. 318. M. Dekker, New York (1980).
41. R. F. Burk and B. S. S. Masters, *Archs. Biochem. Biophys.* **170**, 124 (1975).
42. L. R. Shull, G. W. Buckmaster and P. R. Cheeke, *J. environ. Path. Toxic.* **2**, 1127 (1979).
43. A. Wendel, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), Vol. 1, p. 333. Academic Press, New York (1981).
44. K. Schwarz, *Fedn. Proc.* **24**, 58 (1965).
45. S. G. Jenkinson, R. A. Lawrence, R. F. Burk and D. M. Williams, *J. Nutr.* **112**, 197 (1982).
46. P. D. Whanger, N. D. Pederson and P. H. Weswig, *Biochem. biophys. Res. Commun.* **53**, 1031 (1973).
47. K. P. McConnel, R. M. Burton, T. Kute and P. J. Higgins, *Biochim. biophys. Acta* **588**, 113 (1979).
48. R. F. Burk and P. E. Gregory, *Archs. Biochem. Biophys.* **213**, 73 (1982).
49. A. T. Diplock and J. A. Lucy, *FEBS Lett.* **29**, 205 (1973).
50. R. F. Burk and J. M. Lane, *Fundament. appl. Toxic.* (in press).