Biochemical Pharmacology, Vol. 32, No. 1, pp. 91–96, 1983.
Printed in Great Britain.

0006-2952/83/010091-06 $03.00/0
Pergamon Press Ltd.

DIETARY ASCORBIC ACID AND HEPATIC MIXED FUNCTION OXIDASE ACTIVITY IN THE GUINEA PIG*

FRANCIS J. PETERSON†, DAVID E. HOLLOWAY‡, PETER H. DUQUETTE and JERRY M. RIVERS§

Research Service, Veterans Administration Medical Center, Minneapolis, MN 55417; and Departments of Pharmacology and Medicine, University of Minnesota, Minneapolis, MN 55455; and § Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, U.S.A.

(*Received* 23 *November* 1981; *accepted* 14 *May* 1982)

Abstract—Studies were carried out to characterize the response of hepatic mixed function oxidase (MFO) activity to chronic ascorbic acid deficiency and excessive ascorbic acid intake in the guinea pig. When guinea pigs were fed excessive ascorbic acid, there was a small increase in hepatic cytochrome P-450 which was unaccompanied by any alteration in drug-metabolizing enzyme activity. Similarly, induction of MFO activity by phenobarbital was not modified by excessive ascorbic acid administration. Chronic ascorbic acid deficiency resulted in depressed metabolism of aniline, aminopyrine, ethoxycoumarin and benzphetamine, but not of ethylmorphine, in comparison with animals fed diets containing control and/or excessive amounts of ascorbic acid. In contrast to the metabolism of all drugs studied, the 7α-hydroxylation of cholesterol was depressed by both inadequate and excessive vitamin C intake, demonstrating the unique sensitivity of cholesterol 7α-hydroxylase to dietary ascorbate.

Ascorbic acid deficiency in the guinea pig results in a decrease in the activity of the hepatic microsomal mixed function oxidase (MFO) system [1–11] and in the total content of the terminal oxidase of this system, cytochrome P-450 [4–11]. The biochemical basis for the role of ascorbic acid in drug metabolism is still uncertain although recent studies suggest that the vitamin might be critical for the assembly of the active form of cytochrome P-450 [12].

An unresolved question at present is the dose–response relationship between vitamin C intake and hepatic MFO activity. While the requirement of the guinea pig for ascorbic acid has been determined to be 0.2 g/kg diet or approximately 5 mg/day [13], Zannoni and co-workers [14–16] have reported that supplementation of guinea pigs with doses of vitamin C far in excess of this amount results in a dose-dependent enhancement of hepatic drug metabolism. Additional work by this group demonstrated a positive correlation between hepatic cytochrome P-450 content and liver ascorbic acid concentration [17]. In contrast to these findings, Sikic *et al.* [18], in a study of guinea pigs fed four different levels of dietary ascorbate (0.3, 1.5, 3.5, and 7.0 g/kg diet), failed to observe any dose–response relationship between hepatic ascorbate content and MFO activity or cytochrome P-450 content.

Although numerous studies have described the effects of acute vitamin deprivation on hepatic drug metabolism, no studies to date have documented the influence of chronic ascorbic acid deficiency on this system. The experimental model of chronic ascorbic acid deficiency, characterized and used extensively by Ginter and co-workers [19, 20], offers several advantages over previous studies of drug metabolism in acute vitamin C deficiency: (1) guinea pigs are maintained on intakes of the vitamin adequate to maintain normal rates of growth throughout the experimental period, thereby avoiding secondary complications of scurvy such as inanition, weight loss, and hemorrhage; and (2) this model more closely approximates the clinical situation in human populations of long-term inadequate vitamin C intake unaccompanied by clinical scurvy [21].

In a recent study [22], chronic ascorbic acid deficiency was shown to depress the activity of cholesterol 7α-hydroxylase (EC 1.14.13.17), a cytochrome P-450-dependent enzyme. Depressed enzyme activity was also observed in this study in guinea pigs fed a massive overdose of this vitamin (20 g/kg diet), a dietary intake far in excess of intakes previously studied for effects on MFO activity [14–18]. These observations raise the question of whether other cytochrome P-450-dependent enzymes are similarly altered by dietary extremes of this vitamin. The biologic effects of vitamin C overdosage are of special interest in light of the advocacy of massive intakes of the vitamin for possible health benefits [23].

The present studies were designed to examine the dose–response relationship between ascorbic acid intake and MFO activity and to determine the influence of excessive ascorbic acid administration upon inducibility of the MFO system by phenobarbital. Additionally, an experiment was conducted to elucidate the response of the MFO system to chronic

* This work was supported in part by a grant from Hoffmann-LaRoche, Inc. Nutley, NJ and by the Veterans Administration.

† To whom correspondence should be addressed. Present address: Division of Nutritional Sciences and Department of Poultry Science, Rice Hall, Cornell University, Ithaca, NY 14853, U.S.A.

‡ Present address: Division of Nutritional Sciences, 370 Martha Van Rensselaer Hall, Cornell University, Ithaca, NY 14853, U.S.A.

ascorbic acid deficiency and massive vitamin C overdosage.

MATERIALS AND METHODS

Experimental conditions. Male, weanling guinea pigs (Experiments 1 and 2: English short-hair, Camm Research Institute, Wayne, NJ; Experiment 3: American short-hair, Hill-Top Lab Animals, Scottsdale, PA) were used in all experiments. The animals were fed a cereal-based scorbutigenic diet (Krehl formulation) which has been described previously [22]. The diet was purchased from Teklad Mills, Madison, WI. Food and water were provided *ad lib.* Guinea pigs were housed individually in cages with wire mesh bottoms in rooms subjected to a 12 hr light–dark cycle.

Experiment 1. The first experiment was designed to determine whether intakes of ascorbic acid above the control dose (2 mg per 100 g body wt per day) altered the MFO system. Guinea pigs fed the Krehl diet in pellet form were administered ascorbic acid in a 5% glucose solution for 10 weeks. The ascorbic acid solution was prepared immediately before use and administered orally at doses of 2, 15, 30, and 50 mg/100 g body wt by means of a blunt-tip syringe. The ascorbic acid dose was adjusted every 3 days as the animals gained weight.

Experiment 2. In the second experiment, the influence of excessive ascorbic acid intake upon the metabolism of a variety of substrates and the induction of MFO activity was investigated. Ascorbic acid at doses of 2 and 50 mg per 100 g body wt per day was administered for 10 weeks as described above. At the conclusion of the experimental period, sodium phenobarbital (80 mg/kg body wt) was administered intraperitoneally in a saline solution for 3 days; control animals received saline only. The animals were killed 24 hr after the last injection.

Experiment 3. The final experiment assessed the influences of both inadequate, and excessive, dietary ascorbate on a variety of cytochrome P-450-dependent enzyme activities, including cholesterol 7α-hydroxylase. Guinea pigs were fed powdered diet to which ascorbic acid was added at levels of 50 (deficient), 500 (control), and 20,000 (excess) mg/kg diet. The experimental feeding period was 9 weeks.

Tissue preparation. Animals were stunned by a blow to the head, and blood was obtained by cardiac puncture. A portion of the liver was homogenized in 3 vol. of 1.15% KCl, 50 mM Tris–HCl buffer (pH 7.4) (Experiments 1 and 2) or 100 mM potassium phosphate (pH 7.4) with 150 mM KCl (Experiment 3), using a glass Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 10,000 *g* for 15 min, and the resulting supernatant fraction was centrifuged at 105,000 *g* for 60 min. Microsomes were resuspended in 50 mM Tris–HCl buffer (pH 7.4) (Experiments 1 and 2) or 100 mM potassium phosphate buffer (pH 7.4) (Experiment 3). Microsomal protein was determined by the method of Sutherland *et al.* [24] using crystalline bovine albumin as a standard.

Enzyme assays. Assays of drug-metabolizing enzyme activity were performed aerobically at 37° in 50 mM Tris–KCl buffer (pH 7.4) with saturating concentrations of cofactors and substrates (except in kinetic experiments). Typically, incubations contained the following: microsomes (1 mg/ml), substrate, 0.97 mM $NADP^+$, 5.45 mM glucose-6-phosphate, 5 mM $MgCl_2$, 50 mM Tris (pH 7.4), 150 mM KCl, and 2 units of glucose-6-phosphate dehydrogenase in a total volume of 3 ml. Reactions were run for 10 or 15 min and were terminated with 2 ml of 20% trichloroacetic acid. The kinetics of benzphetamine metabolism were studied using substrate concentrations ranging from 0.05 to 2.0 mM. The *N*-demethylation of benzphetamine, ethylmorphine, and aminopyrine were assayed by measuring the production of formaldehyde using the Nash reagent [25]. The *O*-demethylation of *p*-nitroanisole was measured according to the method of Kato and Gillette [26]. Aniline hydroxylase was determined by the method of Imai *et al.* [27]. The *O*-deethylation of ethoxycoumarin was assayed using the fluorometric procedure described by Ullrich and Weber [28] as modified by Jacobson *et al.* [29]. Cholesterol 7α-hydroxylase activity was measured as described previously [22].

Analysis of ascorbic acid and cytochrome P-450. Ascorbic acid was assayed in trichloroacetic acid extracts of liver, adrenal, spleen and plasma by the method of Roe and Kuether [30] as modified by Bessey [31]. Hepatic microsomal cytochrome P-450 was determined in an Aminco DW-2 spectrophotometer by the method of Omura and Sato [32].

Statistical analysis. The two-tailed Student's *t*-test was used to determine the significance of the differ-

Table 1. Influence of ascorbic acid intake upon hepatic ascorbate concentration, cytochrome P-450 content, and benzphetamine metabolism (Experiment 1)*

Ascorbic acid dose (mg/100 g body wt)	Liver ascorbic acid (mg/100 g)	Hepatic cytochrome P-450 (nmoles/mg protein)	Benzphetamine *N*-demethylase	
			K_m (mM)	V_{max} [nmoles/(min · mg protein)]
2	12.4 ± 0.9[a] (5)†	0.54 ± 0.05[a] (5)	0.26 ± 0.03[a] (3)	2.64 ± 0.19[a] (3)
15	22.4 ± 1.2[b] (5)	0.57 ± 0.05[a] (5)	0.35 ± 0.03[a] (4)	3.00 ± 0.22[a] (4)
30	24.1 ± 1.2[b] (5)	0.63 ± 0.06[a] (5)	0.34 ± 0.02[a] (4)	3.03 ± 0.18[a] (4)
50	25.6 ± 1.6[b] (5)	0.67 ± 0.04[a] (5)	0.38 ± 0.04[a] (3)	3.01 ± 0.20[a] (3)

* Data are expressed as mean ± S.E.M. Means not followed by a common superscript are significantly different ($P < 0.05$). The experimental period was 10 weeks.

† Number of observations.

ence between means with a probability level of $P < 0.05$ considered significant. Regression analysis was carried out by the method of least squares.

RESULTS

The results of Experiment 1 are presented in Table 1. Liver ascorbic acid (AA) concentrations were greater in guinea pigs fed 15, 30, or 50 mg AA/100 g body wt than in animals fed 2 mg AA/100 g body wt, but the hepatic ascorbate levels did not differ significantly among groups receiving 15, 30 or 50 mg AA/100 g body wt. These higher doses of ascorbic acid did not significantly alter either hepatic cytochrome P-450 content or the kinetics of benzphetamine metabolism. However, there was an apparent tendency toward increasing cytochrome P-450 level with increasing ascorbic acid intake. Regression analysis of cytochrome P-450 content versus hepatic ascorbic acid concentration showed a significant relationship between these two variables ($r = 0.55$; $P < 0.05$). These results demonstrate that there was a small but statistically significant increase in cytochrome P-450 content with increasing hepatic ascorbate concentration.

In Experiment 2, consumption of a large surplus of ascorbic acid did not significantly alter the *N*-demethylation of aminopyrine and benzphetamine, the *O*-demethylation of *p*-nitroanisole, or the total content of hepatic microsomal cytochrome P-450 (Table 2). However, once again cytochrome P-450 content was slightly greater in animals receiving large doses of ascorbic acid. Phenobarbital treatment following the 10 weeks of ascorbic acid pretreatment resulted in significant elevations in all microsomal mixed function oxidase activities as compared to their respective controls. Prior consumption of ascorbic acid had little effect on the induction of MFO activity by phenobarbital ip., although *p*-nitroanisole *O*-demethylase was induced by phenobarbital to a greater extent in the excess (50 mg/100 g body wt) group than in controls. Phenobarbital treatment of the excess group caused a significant increase in hepatic ascorbate concentration, while controls exhibited a much smaller effect.

The mean body weights of the animals in Experiment 3, plotted against the days on each experimental diet, are shown in Fig. 1. Despite a 400-fold difference in dietary ascorbate among groups, there was no significant difference in rate of growth. Plasma and tissue ascorbic acid concentrations from Experiment 3 are recorded in Table 3. The three dietary levels of vitamin C resulted in widely different tissue ascorbate values spanning a 17-fold range for hepatic ascorbate and a 21-fold range for plasma ascorbate. The hepatic ascorbic acid concentrations in the deficient guinea pigs in this study were similar to the levels observed in scorbutic animals in studies from other laboratories [33]. However, the guinea pigs in this study did not manifest symptoms of vitamin C deficiency at any time during the experiment.

The influence of ascorbic acid intake on the biotransformation of various substrates by hepatic microsomal enzymes can be seen in Table 4. In agreement with Experiments 1 and 2, guinea pigs

Table 2. Influence of ascorbic acid intake and phenobarbital (PB) treatment on liver ascorbic acid concentration and mixed function oxidase activity (Experiment 2)*

Ascorbic acid dose (mg/100 g body wt)	Liver ascorbic acid (mg/100 g)	*p*-Nitroanisole *O*-demethylase [nmoles/(min · mg protein)]	Aminopyrine *N*-demethylase [nmoles/(min · mg protein)]	Benzphetamine *N*-demethylase K_m (mM)	Benzphetamine *N*-demethylase V_{max} [nmoles/(min · mg protein)]	Hepatic cytochrome P-450 (nmoles/mg protein)
2	14.3 ± 1.2[a]	0.42 ± 0.04[a]	1.98 ± 0.20[a]	0.12 ± 0.01[a]	2.16 ± 0.20[a]	0.56 ± 0.06[a]
2 + PB	16.5 ± 2.0[a]	0.69 ± 0.07[b]	6.99 ± 0.46[b]	0.76 ± 0.04[b]	11.26 ± 0.63[b]	1.07 ± 0.06[b]
50	24.0 ± 1.9[b]	0.39 ± 0.02[a]	2.18 ± 0.19[a]	0.12 ± 0.01[a]	2.36 ± 0.30[a]	0.66 ± 0.05[a]
50 + PB	32.9 ± 1.1[c]	0.87 ± 0.03[c]	6.99 ± 0.52[b]	0.69 ± 0.04[b]	11.13 ± 0.77[b]	1.08 ± 0.04[b]

* Data are expressed as means ± S.E.M. of five animals per group. Means not followed by a common superscript are significantly different ($P < 0.05$). Phenobarbital (i.p.) was given for 3 days (80 mg/kg per day) after 10 weeks of daily ascorbic acid treatment.

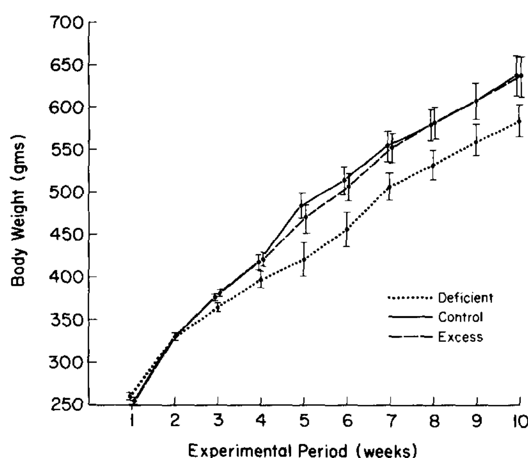

Fig. 1. Rates of growth of guinea pigs fed three dietary levels of ascorbic acid (Experiment 3). Values are means ± S.E.M.

fed excessive ascorbic acid did not differ from control animals in drug-metabolizing enzyme activity. Chronic ascorbic acid deficiency, however, caused significant decreases in the metabolism of aniline, ethoxycoumarin, and benzphetamine. In addition, the rate of aminopyrine metabolism was significantly lower in deficient guinea pigs in comparison with the excess group. Of the five drugs studied, only the metabolism of ethylmorphine was unaffected by ascorbic acid deficiency.

Cytochrome P-450 was lower in the deficient group than in the control and excess groups; however, this difference was statistically significant only between the deficient and excess groups. In agreement with the findings of Experiments 1 and 2, cytochrome P-450 was slightly but not significantly higher in the excess group than in the control group. Regression analysis of cytochrome P-450 content versus hepatic ascorbic acid concentration showed a significant correlation between these two variables (Fig. 2).

Cholesterol 7α-hydroxylase activity was lower in deficient animals than in controls, in agreement with the effects of chronic ascorbic acid deficiency upon most other MFO activities studied. In contrast to drug-metabolizing enzymes, however, cholesterol 7α-hydroxylase was also depressed by excessive ascorbic acid intake. The decrease in the 7α-hydroxylation of cholesterol occurred despite similar cytochrome P-450 levels in control and excess guinea pigs. The absence of an association between cytochrome P-450 content and cholesterol 7α-hydroxylase activity is also evident in comparison of the deficient and excess groups. Despite the fact that cytochrome P-450 content was significantly higher

Table 3. Effects of dietary ascorbic acid on plasma and tissue ascorbic acid levels (Experiment 3)*

Dietary ascorbic acid (g/kg diet)	Ascorbic acid			
	Liver (mg/100 g)	Adrenal (mg/100 g)	Spleen (mg/100 g)	Plasma (mg/dl)
0.05	1.6 ± 0.1[a]	10.8 ± 0.6[a]	5.0 ± 0.3[a]	0.10 ± 0.01[a]
0.50	13.7 ± 0.6[b]	82.6 ± 4.9[b]	36.6 ± 1.6[b]	0.61 ± 0.05[b]
20.0	28.0 ± 1.1[c]	123.0 ± 5.4[c]	45.1 ± 1.6[c]	2.07 ± 0.22[c]

* Data are expressed as means ± S.E.M. of nine to ten animals. Means not followed by a common superscript are significantly different ($P < 0.05$).

The animals were fed their respective diets for 9 weeks.

Table 4. Influence of three levels of dietary ascorbic acid upon hepatic mixed function oxidase activity (Experiment 3)*

	Dietary ascorbic acid		
	0.05	0.50 (g/kg diet)	20.0
Hepatic cytochrome P-450†	0.76 ± 0.03[a]	0.84 ± 0.03[ab]	0.90 ± 0.02[b]
Aniline hydroxylase‡	0.42 ± 0.02[a]	0.55 ± 0.03[b]	0.54 ± 0.02[b]
Aminopyrine *N*-demethylase‡	4.33 ± 0.11[a]	4.61 ± 0.12[ab]	4.79 ± 0.10[b]
Ethoxycoumarin *O*-deethylase§	0.51 ± 0.01[a]	0.77 ± 0.02[b]	0.77 ± 0.03[b]
Benzphetamine *N*-demethylase‡	4.18 ± 0.11[a]	4.58 ± 0.14[b]	4.53 ± 0.15[b]
Ethylmorphine *N*-demethylase‡	3.18 ± 0.16[a]	3.39 ± 0.12[a]	3.18 ± 0.12[a]
Cholesterol 7α-hydroxylase§	1.85 ± 0.19[a]	5.21 ± 0.69[b]	1.18 ± 0.17[c]

* Data are expressed as means ± S.E.M. of nine to ten animals except for cholesterol 7α-hydroxylase where N = five to six. Means not followed by a common superscript are significantly different ($P < 0.05$).

† Expressed as nmoles/mg protein.

‡ Expressed as nmoles/(min · mg protein).

§ Expressed as pmoles/(min · mg protein).

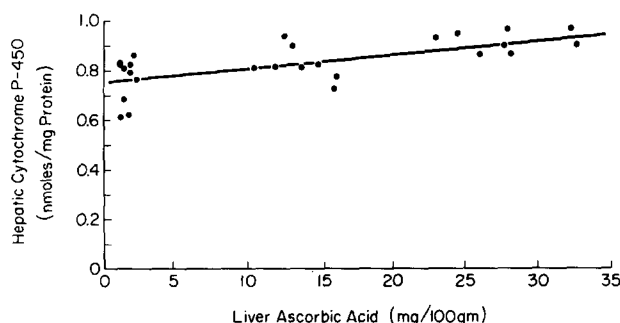

Fig. 2. Correlation between hepatic microsomal cytochrome P-450 content and liver ascorbic acid concentration (Experiment 3). Equation of line: $y = 0.7524 + 0.0055x$; $r = 0.66$, $P < 0.001$.

in the excess group than in the deficient group, excess animals actually had lower cholesterol 7α-hydroxylase activity than deficient animals.

DISCUSSION

The present study demonstrates that chronic ascorbic acid deficiency results in lowered activities of many of the cytochrome P-450-dependent enzymes studied, as well as lower levels of cytochrome P-450 itself. These findings are in agreement with earlier reports of lower MFO activity and cytochrome content in acute ascorbic acid deficiency [1–11]. This report demonstrates that lowered cytochrome P-450 levels and P-450-dependent enzyme activity are specific consequences of vitamin C deprivation since these changes were observed in the absence of secondary complications of acute vitamin C deprivation, e.g. inanition, weight loss, and hemorrhage.

In the present work, components of the MFO system differed in their responses to alteration of ascorbic acid intake. In all three experiments there was a trend toward higher cytochrome P-450 content with increasing dietary ascorbic acid. Increasing dietary ascorbic acid above control levels (2 mg/100 g body wt in Experiments 1 and 2, and 500 mg/kg diet in Experiment 3) resulted in a small increase in cytochrome P-450 level which was not statistically significant in comparisons among groups. However, regression analysis of cytochrome P-450 content versus hepatic ascorbic acid concentration did demonstrate a statistically significant relationship between these variables (Experiments 1 and 3). The biological significance of this rise in cytochrome P-450 content is open to doubt since it is unaccompanied by any increase in drug-metabolizing enzyme activity. The observed lack of effect of excessive ascorbate upon rates of drug metabolism is in agreement with the report of Sikic and co-workers [18] and contrasts with the findings of Zannoni and co-workers [14, 15].

Excessive ascorbic acid intake had a profound effect on one of the microsomal enzymes studied, cholesterol 7α-hydroxylase, in agreement with previous findings [22]. The exceptional sensitivity of this enzyme to vitamin C intake was evident not only with excessive vitamin C intake, in which case the activity decreased 77% while the five drug-metabolizing enzymes studied showed no change, but also with chronic vitamin C deficiency, which resulted in a 64% decrease in activity. Of the five drug-metabolizing enzymes studied, three declined by 9–34% while the other two were unchanged in comparison to control values. Previous studies, employing a variety of conditions, have also documented an anomolous response of cholesterol 7α-hydroxylase relative to other cytochrome P-450-dependent enzymes [34, 35].

The observed divergence of cytochrome P-450 content and the cytochrome P-450-dependent enzyme, cholesterol 7α-hydroxylase, most likely reflects the metabolism of cholesterol by a minor isozyme of cytochrome P-450 which is highly sensitive to ascorbic acid nutriture. A decrease in this isozyme (e.g. in the excess group) would not necessarily be reflected in a decreased total content of cytochrome P-450. Similarly, Kuenzig *et al.* [10] found that 10 days of ascorbic acid depletion decreased the *O*-dealkylation of ethoxycoumarin in the lung and liver of the guinea pig but did not significantly alter cytochrome P-450 content.

Drugs, such as phenobarbital or 3-methylcholanthrene, can stimulate the biotransformation of other chemicals by inducing the microsomal MFO system [36]. Studies of acute ascorbic acid deficiency have demonstrated that deprivation of vitamin C for periods up to 21 days does not alter the ability of drugs, like phenobarbital, to stimulate MFO activity [9]. Similarly, the present study shows that excessive ascorbic acid intake has no generalized effect on the inducibility of the hepatic MFO system by phenobarbital. Although *p*-nitroanisole *O*-demethylase activity was induced to a greater extent in guinea pigs fed excessive ascorbic acid than in controls, no such change was observed for cytochrome P-450 levels or the metabolism of aminopyrine or benzphetamine. Phenobarbital apparently had a different effect on ascorbic acid distribution in guinea pigs fed excessive ascorbic acid than in control animals, as evidenced by the large phenobarbital-induced increase in hepatic ascorbic acid in the excess group. Barbiturates have been shown previously to alter ascorbic acid metabolism in the rat [37].

In summary, the present work demonstrates the ineffectiveness of excessive doses of ascorbic acid in potentiating drug-metabolizing enzyme activity in the guinea pig. The observed small effect of excessive ascorbic acid intake upon hepatic microsomal cytochrome P-450 content is of questionable biological significance since it was unaccompanied by concomitant alteration of enzyme activity. In contrast, chronic vitamin C deficiency altered the metabolism of a variety of substrates, suggesting the usefulness of this model for future work on vitamin C and drug metabolism. Unlike the other enzyme activities studied, cholesterol 7α-hydroxylase was reduced by both inadequate and excessive dietary ascorbate, demonstrating the unique sensitivity of this enzyme to ascorbate nutriture.

Acknowledgements—We wish to express our gratitude to Mrs. Esserlene Gatewood, Mr. Richard Erickson, and Mr. Jeremy Holtzman for excellent technical assistance.

REFERENCES

1. R. K. Richards, K. Kueter and T. J. Klatt, *Proc. Soc. exp. Biol. Med.* **48**, 403 (1941).
2. J. Axelrod, S. Udenfriend and B. B. Brodie, *J. Pharmac. exp. Ther.* **111**, 176 (1954).
3. A. H. Conney, G. A. Bray, C. Evans and J. J. Burns, *Ann. N.Y. Acad. Sci.* **92**, 115 (1961).
4. R. Kato, A. Takanaka and T. Oshima, *Jap. J. Pharmac.* **19**, 25 (1969).
5. E. Degkwitz, D. Luft, U. Pfeiffer and H. Staudinger, *Hoppe-Seyler's Z. physiol. Chem.* **349**, 465 (1968).
6. E. Degkwitz, L. Hochli-Kaufmann, D. Luft and H. Staudinger, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1023 (1972).
7. A. E. Wade, B. Wu and P. B. Smith, *J. pharm. Sci.* **61**, 1205 (1972).
8. V. G. Zannoni, E. J. Flynn and M. Lynch, *Biochem. Pharmac.* **21**, 1377 (1972).
9. V. G. Zannoni and M. Lynch, *Drug Metab. Rev.* **2**, 57 (1973).
10. W. Kuenzig, V. Tkaczevski, J. J. Kamm, A. H. Conney and J. J. Burns, *J. Pharmac. exp. Ther.* **201**, 527 (1977).
11. B. I. Sikic, E. G. Mimnaugh, C. L. Litterst and T. E. Gram, *Archs Biochem. Biophys.* **179**, 663 (1977).
12. J. D. Turnbull and S. T. Omaye, *Biochem. Pharmac.* **29**, 1255 (1980).
13. National Research Council, *Nutrient Requirements of Domestic Animals. 10. Nutrient Requirements of Laboratory Animals*, pp. 59–69. National Academy of Sciences, Washington, DC (1978).
14. P. H. Sato and V. G. Zannoni, *Biochem. Pharmac.* **23**, 3121 (1974).
15. V. G. Zannoni and P. H. Sato, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds. P. L. Morselli, S. Garratini and F. Sereni), p. 301. Raven Press, New York (1975).
16. V. G. Zannoni and P. H. Sato, *Ann. N.Y. Acad. Sci.* **258**, 119 (1975).
17. L. E. Rikans, C. R. Smith and V. G. Zannoni, *J. Pharmac. exp. Ther.* **204**, 702 (1978).
18. B. I. Sikic, E. G. Mimnaugh and T. E. Gram, *Biochem. Pharmac.* **26**, 2037 (1977).
19. E. Ginter, P. Bobek and M. Ovecka, *Int. J. Vitam. Res.* **38**, 104 (1968).
20. E. Ginter, R. Nemec, J. Cerven and L. Mikus, *Lipids* **8**, 135 (1973).
21. M. L. Burr, P. C. Elwood, D. J. Hole, R. J. Hurley and R. E. Hughes, *Am. J. clin. Nutr.* **27**, 144 (1974).
22. D. E. Holloway and J. M. Rivers, *J. Nutr.* **111**, 412 (1981).
23. L. Pauling, *Vitamin C and the Common Cold*. W. H. Freeman, San Francisco (1970).
24. E. W. Sutherland, C. F. Cori, R. Haynes and N. S. Olsen, *J. biol. Chem.* **180**, 825 (1949).
25. T. Nash, *Biochem. J.* **55**, 416 (1953).
26. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
27. Y. Imai, A. Ito and R. Sato, *J. Biochem., Tokyo* **60**, 417 (1966).
28. V. Ullrich and P. Weber, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1171 (1972).
29. M. Jacobson, W. Levin, P. J. Poppers, A. W. Wood and A. H. Conney, *Clin. Pharmac. Ther.* **16**, 701 (1974).
30. J. H. Roe and C. A. Kuether, *J. biol. Chem.* **147**, 399 (1943).
31. O. A. Bessey, in *Vitamin Methods* (Ed. P. Gyorgy), Vol. 1, p. 303. Academic Press, New York (1950).
32. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
33. W. Kuenzig, R. Avenia and J. J. Kamm, *J. Nutr.* **104**, 952 (1974).
34. J. E. Gielen, J. Van Cantfort and P. Kremers, *Archs Toxic.* **36**, 255 (1976).
35. W. S. Mellon, D. T. Witiak and D. R. Feller, *Biochem. Pharmac.* **27**, 1055 (1978).
36. F. J. Peterson and J. L. Holtzman, in *Extrahepatic Metabolism of Drugs and Other Foreign Compounds* (Ed. T. E. Gram), p. 1. Spectrum, New York (1980).
37. J. J. Burns, E. H. Mosbach and S. Schulenberg, *J. biol. Chem.* **207**, 679 (1954).