EFFECTS OF ASCORBIC ACID ON MICROSOMAL DRUG METABOLISM *

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Studies from a number of laboratories have shown that vitamin C deficiency in guinea pigs results in decreased metabolism of a variety of pharmacological agents.[1-10] In 1941, Richards et al.[4] found a prolonged sleeping time in scorbutic guinea pigs given pentobarbital compared with that of normal animals, and the administration of ascorbic acid reversed the effect. Axelrod et al.[5] in 1954 reported up to a threefold increase in plasma half-life of such agents as acetanilid, aniline, or antipyrine in guinea pigs depleted of vitamin C that was attributed to lowered rates of metabolism. In addition, the increase in plasma half-life of these agents could be reversed in 4 to 6 days if the diet was replenished with ascorbic acid. Other laboratories[6, 7] have reported decreased oxidation of such agents as zoxazolamine, acetanilid, coumarin, diphenylhydramine, and meperidine in microsomes prepared from scorbutic guinea pigs. Our current knowledge of the hepatic electron transport system involving NADPH, cytochrome P-450 reductase, cytochrome P-450, and molecular oxygen required for the mixed-function oxygenase-type drug hydroxylation reactions has allowed a more direct approach in studies on the effect of ascorbic acid in the oxidation of a variety of pharmacological agents.

Results

If groups of adult guinea pigs weighing 250–400 grams were maintained on a vitamin-C-deficient diet for 21 days, the hydroxylation of aniline, aminopyrine, and *p*-nitroanisole as well as the quantity of cytochrome P-450 and cytochrome P-450 reductase was significantly decreased; the liver microsomal ascorbic acid was 30% of normal; the animals had lost at most 5% of their body weight and were not frankly scorbutic. On the other hand, if adult guinea pigs were maintained on a vitamin-C-deficient diet for only 10 days, there was no significant difference in drug metabolism (TABLE 1). In addition, study of groups of fasted guinea pigs that had lost up to 30% of their body weight indicated that the decreased drug metabolism observed in the 21-day vitamin-C-deficient animals could not be due to the weight loss observed in this group (5%) since in the starved animals drug metabolism was either normal or increased two- to threefold.[3] These studies as well as those from other laboratories were done in the main with adult guinea pigs severely depleted of ascorbic acid.[5, 7, 8, 10, 13] It was, therefore, important to determine if weanling guinea pigs (90 to 100 g

* This study was supported by Grant 17184 from the National Institutes of Health, United States Public Health Service and Grant 23007 from Hoffmann-LaRoche, Inc.

and 1 to 2 weeks of age) placed on a vitamin-C-deficient diet for a relatively short period (8 or 15 days) would be more dependent on the vitamin to maintain adequate drug metabolism, since they are in their rapid period of growth. In addition, weanling guinea pigs were given high supplements of vitamin C (up to 75 mg per day) to observe what effect excess ascorbic acid, well beyond the recommended daily requirement, would have on the liver microsomal-drug-metabolizing system. The data in FIGURE 1 illustrate the increasing pattern of activity of NADPH-cytochrome P-450 reductase in groups of weanling guinea pigs on a deficient diet for 15 days (point 1), those on a deficient diet for 8 days, and those given extra ascorbic acid, up to 75 mg per day. The activity of NADPH-cytochrome P-450 reductase increased almost threefold over that of 15-day-vitamin-C-deficient animals when the liver ascorbic acid concentration was 27.0 mg/100 g liver; there was a twofold increase over that of 8-day deficient animals. A similar pattern of increase in the quantity of cytochrome P-450 was found with increasing *in vivo* administration of ascorbic acid but was smaller in magnitude. At a liver ascorbic acid concentration of 27 mg/100

TABLE 1

EFFECT OF VITAMIN C DEFICIENCY (10 AND 21 DAYS) ON DRUG ENZYMES AND ELECTRON-TRANSPORT COMPONENTS IN GUINEA PIG LIVER MICROSOMES

	Activity *			
	Normal	Vitamin-C-deficient (10 Days)	Vitamin-C-deficient (21 Days)	Decrease (%)
Aniline hydroxylase	1.6 ±0.02	1.3 ±0.1	0.8 ±0.2 $p < 0.001$	50
Aminopyrine *N*-demethylase	3.9 ±0.1	3.3 ±0.4	1.7 ±0.3 $p < 0.001$	56
p-Nitroanisole *O*-demethylase	3.2 ±0.4	3.0 ±0.2	1.1 ±0.2 $p < 0.001$	66
Cytochrome P-450	0.05±0.01	0.05±0.001	0.03±0.003 $p < 0.01$	40
NADPH cytochrome P-450 reductase	0.80±0.2	0.87±0.33	< 0.10	85
NADPH cytochrome *c* reductase	124±21	167±20	83±11 $p < 0.05$	33
Cytochrome b_5	0.03±0.004	0.03±0.003	0.02±0.006 $p < 0.05$	33
Liver ascorbic acid	19.4 ±2.9	6.2 ±1.5	2.5 ±1.5	—
Supernatant fraction—15,000×*g* (mg/100 g wet weight)				
Microsomal fraction (mg/100 g wet weight)	1.0 ± .38	0.6 ±0.09	0.35±0.20	—

* Activity of aniline hydroxylase, aminopyrine, *N*-demethylase, and *p*-nitroanisole, *O*-demethylase equals μmoles of product formed/hr/100 mg of microsomal protein at 27°. Cytochrome P-450 equals μmoles/100 mg microsomal protein; NADPH cytochrome P-450 reductase equals μmoles reduced/hr/100 mg microsomal protein at 27° C. Mean ± SE of 10 animals per group.[3]

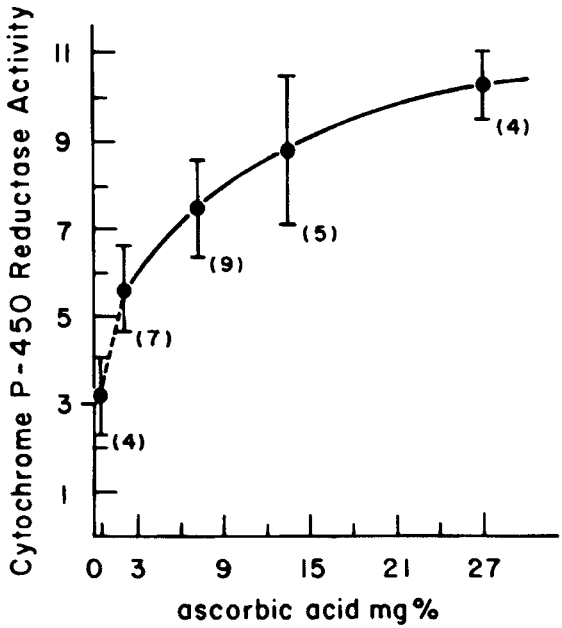

FIGURE 1. NADPH-cytochrome P-450 reductase activity in weanling guinea pigs given varying amounts of ascorbic acid. Specific activity is expressed as μmoles cytochrome P-450 reduced/hr/100 mg of microsomal protein at 27°. The number of animals in each group is given in the FIGURE. The diets of the various groups contained: (1) less than 1.0 mg ascorbic acid/100 g liver, ascorbic-acid-deficient diet for 15 days; (2) 2.3 mg ascorbic acid/100 g liver, ascorbic-acid-deficient diet for 8 days; (3) 7.4 mg ascorbic acid/100 g liver, 25 mg day of ascorbic acid given for 8 days; (4) 13.5 mg ascorbic acid/100 g liver, 75 mg day of ascorbic acid given for 8 days; and (5) 27.0 mg ascorbic acid/100 g liver, chow guinea pig diet plus greens given 8 days. (From Sato and Zannoni.[15] By permission of *Biochemical Pharmacology*.)

g there was 0.068 μmoles of cytochrome P-450/100 mg of microsomal protein as against 0.047 in animals on a deficient diet for 15 days. The data in FIGURE 2 show a substantial increase in *p*-nitroanisole *O*-demethylase activity with increasing administration of ascorbic acid to weanling guinea pigs. There was a fourfold increase in *O*-demethylation at a liver ascorbic acid concentration of 27 mg per 100 g over that of the 15-day-deficient group (liver ascorbic acid; <1.0 mg/100 g liver) and approaching a twofold increase over that of the 8-day deficient group (liver ascorbic acid; 2.7 mg/100 g liver). A similar pattern of increase was observed in *N*-demethylase activity but was not as striking as that observed with *O*-demethylation. There was a 60% increase in the *N*-demethylation of aminopyrine in animals with a liver ascorbic acid concentration of 27 mg/100 g over that of the 15–day deficient group. It should be mentioned that the body weights of the animals on a deficient diet for 8 or 15 days did not significantly differ from the body weights of the control groups of animals, i.e., normal chow diet supplemented with greens 3 times a week. Also, there was no signs of scurvy in the 8-day deficient group, while some of

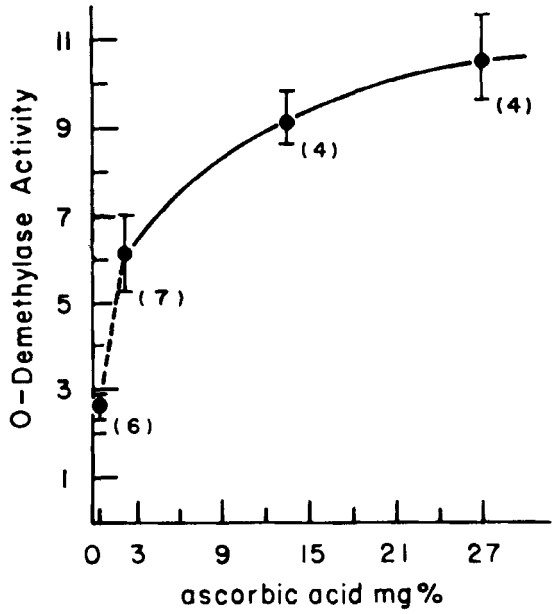

FIGURE 2. *p*-Nitroanisole *O*-demethylase activity in weanling guinea pigs given varying amounts of ascorbic acid. Specific activity is expressed as μmoles *p*-nitrophenol formed/hr/100 mg of microsomal protein at 27°. The number of animals in each group is shown in the figure. The diets of the various groups contained: (1) less than 1.0 mg ascorbic acid/100 g liver, ascorbic-acid-deficient diet given for 15 days; (2) 2.7 mg ascorbic acid/100 g liver, ascorbic-acid-deficient diet given for 8 days; (3) 13.5 mg ascorbic acid/100 g liver 75 mg/day of ascorbic acid given for 8 days; and (4) 27.0 mg ascorbic acid/100 g liver, chow guinea pig diet plus greens given for 8 days. (From Sato and Zannoni.[15] By permission of *Biochemical Pharmacology*.)

the animals in the 15-day deficient group had moderate joint hemorrhages. It should be noted that there was no significant difference in the amount of decreased drug-metabolizing enzyme activites in the 15-day deficient animals with or without hemorrhages.

Further studies with fetal guinea pig livers indicated a marked variation in cytochrome P-450 and *O*-demethylase activity in individual fetal livers, which correlated well with the concentration of liver ascorbic acid (TABLE 2). Fetal livers with an ascorbic acid concentration of below 5.0 mg/100 g liver had no detectable cytochrome P-450 or *O*-demethylase activity while fetal livers with high ascorbic acid levels (above 17.0 mg/100 g liver) had much higher drug metabolism activity; cytochrome P-450 concentration, 84% of the dams; and *O*-demethylase activity, 51% of the dams.

Specificity studies were carried out to determine if other reducing agents, such as reduced 2,6-dichlorophenolindophenol dye, reduced glutathione, and D-isoascorbic acid, would be effective in increasing overall liver microsomal drug-metabolizing reactions. These compounds were fed to groups of weanling guinea pigs on an ascorbic-acid-deficient diet for 8 days. In addition, ascorbyl

palmitate, a more lipophilic analogue of ascorbic acid, was used to determine if it would be more effective than ascorbic acid in increasing drug-metabolism activities (TABLE 3). Reduced 2,6-dichlorophenolindophenol dye (2 mg/day) did not significantly affect the liver microsomal levels of cytochrome P-450 and NADPH cytochrome P-450 reductase or the overall microsomal drug-oxidation reaction, *p*-nitroanisole *O*-demethylation. Aminopyrine *N*-demethylation, however, was somewhat increased compared with that of the deficient groups. The administration of reduced glutathione to guinea pigs (10 mg/day) did not significantly alter liver microsomal cytochrome P-450 concentration or *p*-nitroanisole *O*-demethylase activity from those values in 8-day ascorbic-acid-deficient animals. There was, however, an increase in aminopyrine *N*-demethylase activity in this group and an increase of 59% in NADPH cytochrome P-450 reductase activity over the activities in 8-day vitamin-C-deficient guinea pigs. D-isoascorbic acid (200 mg/day) was not effective in increasing either drug-metabolism activities or electron-transport components. In comparison, ascorbyl palmitate was more effective in increasing levels of electron transport components and overall drug-oxidation reactions. Administration of 50 mg/day, 0.12 mM, led to a liver ascorbic acid concentration of 10.8 mg%, whereas administration of 0.31 mM of ascorbic acid was required to obtain a comparable liver ascorbic acid concentration, i.e., 13.5 mg%. The concentration of cytochrome P-450 was as high as that in the group of animals on a normal chow diet.

Reversal of decreased drug metabolism activities in vitamin-C-deficient animals by the *in vivo* administration of ascorbic acid indicated that, although the quantity of liver ascorbic acid was restored to normal levels within 3 days, most of the drug enzyme activities required 6 to 10 days of ascorbic acid administration to return to normal. Aniline hydroxylase activity required from 3 to 6 days, while aminopyrine *N*-demethylase and *p*-nitroanisole *O*-demethylase activities returned to normal levels within 6 to 10 days (FIGURE 3). Microsomal electron transport components, such as cytochrome P-450 and NADPH cytochrome P-450 reductase, also returned to normal levels within 6 to 10 days (FIGURE 4).

TABLE 2

CYTOCHROME P-450, *O*-DEMETHYLASE AND ASCORBIC ACID LEVELS IN FETAL GUINEA PIG LIVERS

	Ascorbic Acid mg/100 g Liver	P-450 μmoles/100 mg Microsomal Protein	*O*-Demethylase μmoles Product Formed/hr/100 mg Microsomal Protein at 27°
fetal (2)*	4.5	< 0.001	< 0.01
fetal (3)*	11.2	0.004	0.11
fetal (4)*	17.5	0.011	0.78
dam (3)	21.7	0.013	1.73

* 64–66 days of gestation, number in parentheses equals numbers of animals; average values given; ascorbic acid determined by method of Zannoni *et al.*[16] Individual values in groups did not differ by more than 12%.

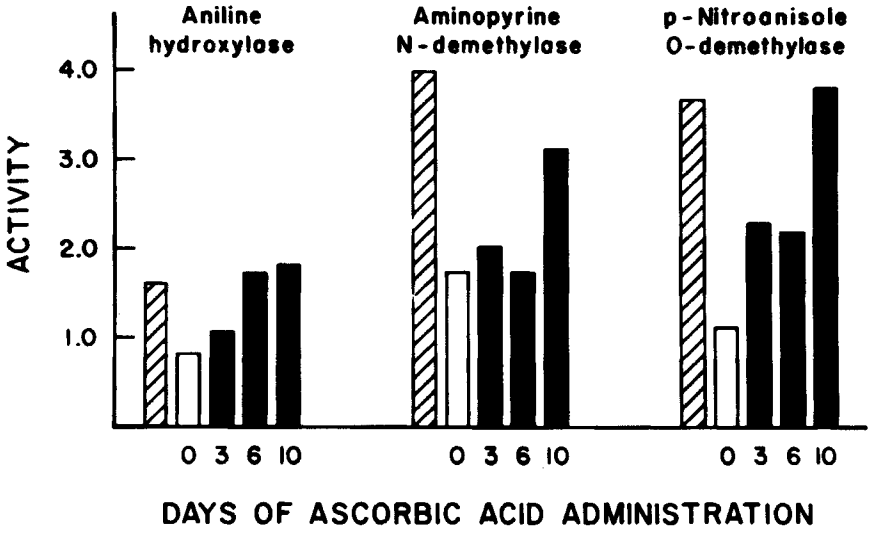

FIGURE 3. Reversal of decreased aniline hydroxylase, aminopyrine *N*-demethylase, and *p*-nitroanisole *O*-demethyalse activities in vitamin-C-deficient guinea pigs with ascorbic acid. Groups of normal and vitamin-C-deficient guinea pigs (21 days) were given 50 mg of ascorbic acid in their drinking water for 3, 6 and 10 days. Enzyme activity equals the micromoles product formed per hours per 100 mg liver microsomal protein at 27°. (From Zannoni *et al.*[3] By permission of *Biochemical Pharmacology*.)

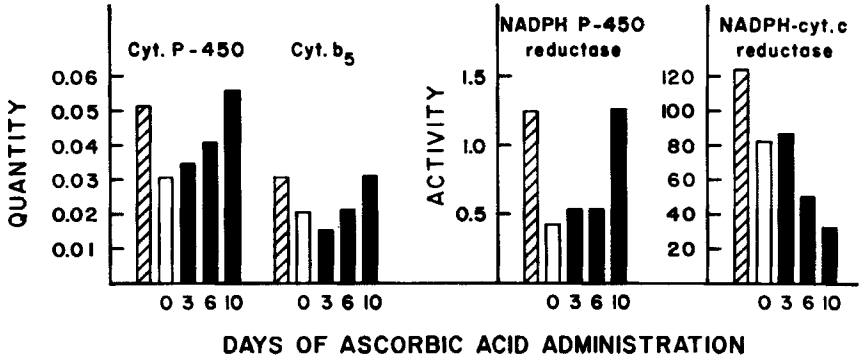

FIGURE 4. Reversal of decreased electron-transport components in vitamin-C-deficient guinea pigs with ascorbic acid. Groups of normal and vitamin-C-deficient guinea pigs (21 days) were given 50 mg of ascorbic acid in their drinking water for 3, 6 and 10 days. The quantity of cytochrome b_5 equals the micromoles per 100 mg liver microsomal protein. NADPH cytochrome P-450 reductase equals the micromoles of cytochrome P-450 reduced per hour per 100 mg liver microsomal protein at 27°. NADPH cytochrome *c* reductase equals the micromoles of cytochrome *c* reduced per hour per 100 mg liver microsomal protein at 27°. Cytochrome P-450 equals the micromoles per 100 mg liver microsomal protein. (From Zannoni *et al.*[3] By permission of *Biochemical Pharmacology*.)

Other studies using phenobarbital indicated that overall drug oxidation activites (aniline hydroxylase, aminopyrine *N*-demethylase, and *p*-nitroanisole *O*-demethylase) and microsomal electron transport components were induced in vitamin-C-deficient guinea pigs comparable to that found in normal animals (TABLE 4). Aniline hydroxylase increased 2.1–fold in the vitamin-C-deficient animal as against 1.4–fold in the normal animal, and *p*-nitroanisole *O*-demethylase increased 5.9–fold in the vitamin-C-deficient animal compared to 3.5–fold in the normal animal. Furthermore, the difference between the level of activity after phenobarbital treatment and basal level (no treatment) in vitamin-C-

TABLE 3

EFFECT OF VARIOUS REDUCING AGENTS IN GUINEA PIG DRUG METABOLISM, *In Vivo*

Diet *	Liver Ascorbic Acid (mg%)	Cytochrome P-450	NADPH Cytochrome P-450 Reductase †	*p*-Nitroanisole *O*-Demethylase †	Aminopyrine *N*-Demethylase
Chow diet	27.0	0.070	10.3	10.6	15.7
Ascorbic-acid-deficient diet	2.0	0.060	5.6	6.0	10.8
+ascorbate	13.5	0.067	8.7	9.1	15.6
+reduced 2,6-DCPP	3.8	0.056	6.0	5.4	13.5
+reduced GSH	1.2	0.064	8.9	6.0	13.6
+D-isoascorbate	5.7	0.058	4.6		11.3
+ascorbyl palmitate	10.8	0.069	6.7	8.3	13.0

* Treatment: Groups of weanling guinea pigs were fed on a chow guinea pig diet supplemented with greens and an eight-day ascorbic-acid-deficient guinea pig diet. Reduced 2,6-dichlorophenolindophenol dye, 2 mg/day, given to guinea pigs on ascorbic-acid-deficient diet, treated for 8 days. Reduced glutathione, 10 mg/day, given to guinea pigs on ascorbic-acid-deficient diet, treated for 8 days. D-isoascorbate, 200 mg/day, given to guinea pigs on ascorbic-acid-deficient diet, treated for 8 days. Ascorbyl palmitate, 50 mg/day, supplemented to guinea pigs on ascorbic-acid-deficient diet, treated for 8 days.[4]

† Activities of cytochrome P-450, P-450 reductase, *O*-demethylase and *N*-demethylase equals μmoles of product formed/hr/100 mg of microsomal protein at 27° C.

deficient and normal guinea pigs was in the same order of magnitude for each enzyme activity. The individual electron-transport components were also induced in vitamin-C-deficient guinea pigs and the fold increase was equivalent to, or in some cases better than, that of normal guinea pigs.

It was of interest to determine if changes in kinetic constants occurred in weanling guinea pigs made vitamin-C-deficient or, more important, if any alteration in the apparent affinity occurred in animals under conditions of increased drug-metabolism activities, such as when large amounts of ascorbic acid were administered. Apparent affinity constants of aminopyrine *N*-demethylase were determined with microsomes isolated from 8- and 15-day-deficient animals,

animals receiving 50 or 500 mg per day of ascorbic acid for 8 days, and in guinea pigs given a normal diet of chow pellets. Under these conditions the animals had decreased as well as increased drug enzyme activity. FIGURE 5 gives the kinetic data from which the K_M of liver microsomal aminopyrine *N*-demethylase was calculated for 8- and 15-day ascorbic-acid-deficient animals, and for animals given the normal diet. In contrast to the differences in aminopyrine *N*-demethylase activities found in these groups, the Michaelis-Menten affinity constants were not significantly altered. The K_M value for normal weanling guinea pigs was 1.67×10^{-3} M. Eight- and 15-day-deficient animals had K_M values of 1.67×10^{-3} M, and 1.89×10^{-3} M respectively. FIGURE 6 gives the kinetic data from which the K_M of liver microsomal aminopyrine

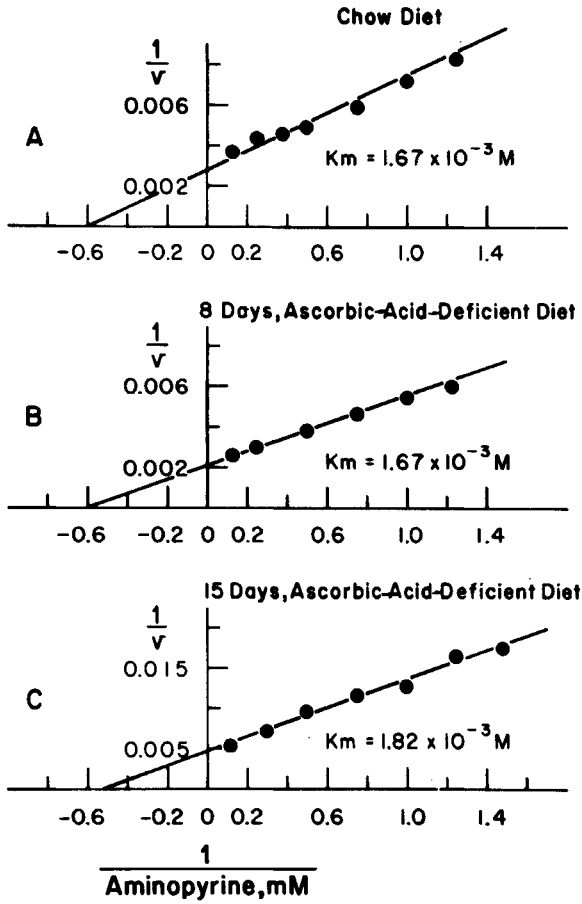

FIGURE 5. Apparent K_M of liver microsomal aminopyrine *N*-demethylase activity in weanling guinea pigs on various ascorbic acid regimens. On chow diet plus greens liver ascorbic acid is 27 mg/100 g liver. On 8-day ascorbic-acid-deficient diet liver ascorbic acid is 2 mg/100 g liver. On 15-day ascorbic-acid-deficient diet liver ascorbic acid is less than 1 mg/100 g liver.

TABLE 4

PHENOBARBITAL INDUCTION OF DRUG ENZYMES AND ELECTRON-TRANSPORT COMPONENTS IN NORMAL AND VITAMIN-C-DEFICIENT GUINEA PIGS

	Activity *					
	Normal			Vitamin-C-Deficient		
	No R_x	PB R_x†	Fold Increase	No R_x	PB R_x†	Fold Increase
Aniline hydroxylase	1.6	2.3	1.4	0.8	1.7	2.1
Aminopyrine *N*-demethylase	3.9	9.6	2.5	1.7	7.6	4.5
p-Nitroanisole *O*-demethylase	3.2	11.1	3.5	1.1	6.5	5.9
Cytochrome P-450	0.05	0.11	2.2	0.03	0.06	2.0
NADPH P-450 reductase	0.8	2.6	3.3	<0.10	2.8	28.0
NADPH cytochrome *c* reductase	124	288	2.3	83	250	3.0
Cytochrome b_5	0.03	0.03	1.0	0.02	0.02	1.0

* Activity equals μmoles of product formed/hr/100 mg of microsomal protein at 27° C. Liver ascorbic acid: normal induced, 195 μg/g wet weight (15,000×*g* supernatant fraction), 19 μg/g wet weight (microsomal fraction); vitamin-C-deficient induced, 71 μg/g wet weight (15,000×*g* supernatant fraction), 5 μg/g wet weight (microsomal fraction).[2]

† Phenobarbital treatment: guinea pigs received 1 mg sodium phenobarbital/ml for 4 days in drinking water; 30 to 40 ml consumed per day.

N-demethylase was calculated for weanling guinea pigs given 50 mg and 500 mg ascorbic acid per day for 8 days, and for animals maintained on a normal chow diet. As can be observed, there was no significant difference in these values. The K_M value for animals given 50.0 mg per day of ascorbic acid was 1.57×10^{-3} M. Animals receiving 500 mg per day of ascorbic acid for the 8-day period had an affinity constant of 1.85×10^{-3} M. The K_M value of aminopyrine *N*-demethylase in weanling guinea pigs given a chow diet was 1.67×10^{-3} M. Similar results were obtained in kinetic studies with *O*-demethylase in that there was no significant alteration in the K_M constants for this enzyme system.

Conclusions and Summary

Both *in vivo* and *in vitro* investigations from several laboratories have shown that vitamin-C deficiency results in decreased metabolism of a variety of pharmacological agents. These studies have demonstrated that *O*-demethylation, *N*-demethylation, and hydroxylation reactions as well as individual liver microsomal electron transport components such as cytochrome P-450 and NADPH cytochrome P-450 reductase are decreased in guinea pigs depleted of ascorbic acid. In addition, the decreased drug enzyme activities can be restored to normal levels if the deficient animals are given the vitamin for a period of 6 to 10 days. The majority of these previous studies were carried out in adult guinea pigs (250–400 g) maintained on a deficient diet for a substantial length of time, up to 21 days.

Our more recent studies include the effect of ascorbic acid on drug metabolism in weanling animals maintained for a short period on a vitamin-C-deficient diet (8–15 days); these animals were used since they are in their rapid growth period and are more susceptible to a vitamin deficiency. Importantly, these

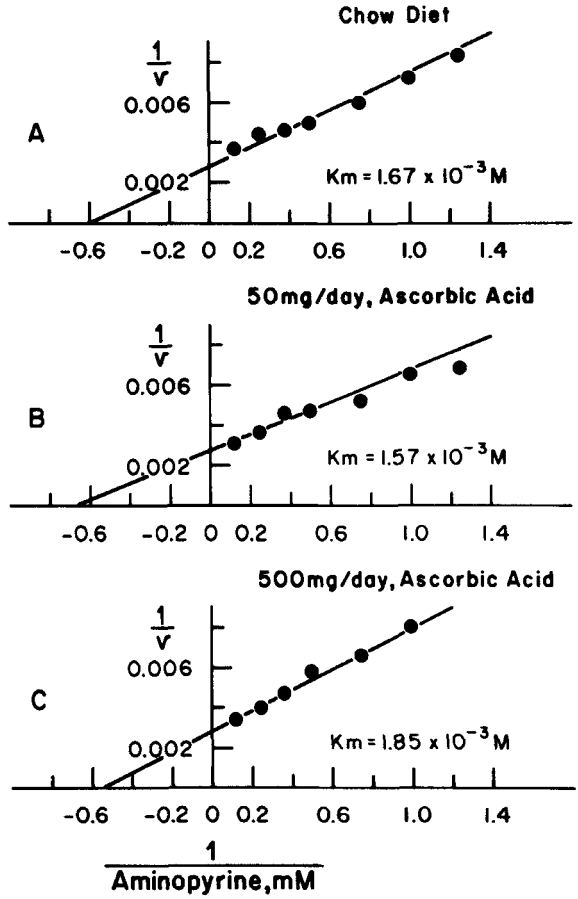

FIGURE 6. Apparent K_M of liver microsomal aminopyrine *N*-demethylase activity in weanling guinea pigs on various ascorbic acid regimens. On chow diet plus greens liver ascorbic acid is 27 mg/100 mg liver. On 50 mg per day ascorbic acid for 8 days liver ascorbic acid is 9.9 mg/100 g liver. On 500 mg per day ascorbic acid for 8 days liver ascorbic acid is 23.3 mg/100 g liver.

studies indicate that not only does deficiency result in decreased drug metabolism, but also the activity of the microsomal drug oxidation system increases over basal levels when the animals are given high supplements of the vitamin. NADPH cytochrome P-450 reductase, *N*-demethylase, and *O*-demethylase activities increased as much as 200% while the increase in the quantity of cyto-

chrome P-450 was smaller and indicates that the cytochrome may not be rate-limiting with respect to the effect of ascorbic acid. In contrast, cytochrome P-450 reductase activity markedly increased and showed a greater dependence on the liver concentration of ascorbic acid since the increases observed correlated well with the amount of ascorbic acid present in the liver. Furthermore, our studies with fetal guinea pig liver indicate a marked reduction in overall drug metabolism reactions and quantity of electron-transport components in liver with low concentrations of ascorbic acid. Comparison of drug metabolism activities with liver ascorbic acid concentrations in individual fetal livers showed a significant correlation of drug metabolism ability and ascorbic acid concentration. Some of the delayed appearance of drug-enzyme activity known to occur in prenatal guinea pigs could be due, in part, to the amount of ascorbic acid present in the fetal liver. It would be of interest to compare various drug and steroid hydroxylation systems at various times of gestation during the fetal life of the guinea pig with ascorbic acid levels as well as with the changes in the hepatic smooth endoplasmic reticulum in the developing fetus.[11, 12] With respect to this, protein synthesis involved in the electron-transport system in the adult guinea pig is not affected in vitamin-C deficiency in that induction occurs with phenobarbital to the same extent as in normal animals.[3]

Specificity studies with analogues of ascorbic acid indicate that the stimulation of drug enzyme activities occurs with such compounds as D-isoascorbic acid or ascorbyl palmitate. However, much higher amounts of D-isoascorbic acid were required than of the vitamin, which can be explained in part by the difficulty in obtaining liver concentrations of the D-isomer equivalent to those of vitamin C, since the former is rapidly excreted by the kidney. On the other hand, ascorbyl palmitate, a more lipophilic compound than the vitamin, was as effective as ascorbic acid on a molar basis.

To date, our studies [3] as well as those of other investigators,[8, 13] have indicated that the quality as well as the quantity of the cytochrome P-450 is altered in vitamin-C-deficient microsomes. Both type I and type II substrate binding spectra are atypical, with microsomes isolated from vitamin-C-deficient animals in that the peak, trough, and magnitude of absorption are altered. Changes in cytochrome P-450 substrate binding spectra may indicate an alteration in the structure of the hemeprotein. For that matter, the atypical binding spectra in vitamin-C-deficient guinea pig microsomes may reflect an alteration in the essential phospholipid moiety needed for functioning cytochrome P-450.

Further *in vitro* studies on the purification and separation of individual microsomal electron-transport components from normal and vitamin-C-deficient livers will be helpful in ascertaining a more precise biochemical understanding of the function of ascorbic acid in drug metabolism. Qualitative physical-chemical differences in addition to quantitative differences in electron-transport components such as cytochrome P-450, differences in substrate P-450 binding, and possible alteration in the important microsomal phospholipid moiety could be investigated. In addition, kinetic studies with the component partial reactions involved in drug hydroxylation would be helpful in determining any stoichiometric requirements of ascorbic acid for maximum drug hydroxylation as well as any interaction of ascorbic acid with particular microsomal components. Concomitant with these studies, experiments concerned with the synthesis and degradation of cytochrome P-450 or an altered form of the cytochrome as well as studies on the turnover of the essential phospholipid associated with the microsomal electron-transport system in normal and vitamin-C-deficient guinea pigs would be of interest.

The requirement of vitamin C in the metabolism of drugs in man may be of clinical importance, especially in the young. In this respect the recommended amount of the vitamin, 70–75 mg per day [14] may not be adequate for optimum activity of the microsomal drug enzymes, especially if an increased formation of metabolites followed by subsequent conjugation reactions are necessary to ensure efficient detoxification. It would be of interest to determine if increased intake of ascorbic acid in man, above the daily recommended requirements, would decrease the biological plasma half-life of a variety of commonly used drugs.

References

1. ZANNONI, V. G. & M. M. LYNCH. 1973. The role of ascorbic acid in drug metabolism. Drug Metab. Rev. **2:** 57–69.
2. ZANNONI, V. G., M. M. LYNCH & P. H. SATO. 1974. *In* Perinatal Pharmacology: Problems and Priorities. J. Dancis & J. C. Hwang, Eds. 131–147. Raven Press. New York, N.Y.
3. ZANNONI, V. G., E. J. FLYNN & M. LYNCH. 1972. Ascorbic acid and drug metabolism. Biochem. Pharmacol. **21:** 1377–1392.
4. RICHARDS, R. K., K. KUETER & T. I. KLATT. 1941. Effect of vitamin C deficiency on action of different types of barbiturates. Proc. Soc. Exp. Biol. Med. **48:** 403–409.
5. AXELROD, J., S. UDENFRIEND & B. B. BRODIE. 1954. Ascorbic acid in aromatic hydroxylation. III. Effect of ascorbic acid on hydroxylation of acetanilide, aniline and antipyrine *in vivo*. J. Pharmacol. Exp. Ther. **111:** 176–181.
6. CONNEY, A. H., G. A. BRAY, C. EVANS & J. J. BURNS. 1961. Metabolic interactions between L-ascorbic acid and drugs. Ann. N.Y. Acad. Sci. **92:** 115–127.
7. KATO, R., A. TAKANAKA & T. OSHIMA. 1969. Effect of vitamin C deficiency on metabolism of drugs and NADPH-linked electron transport system in liver microsomes. Jap. J. Pharmacol. **19:** 25–33.
8. GUNDERMANN, K., E. DEGKWITZ & H. STAUDINGER. 1973. Mischfunktionelle oxygenierung von (+) und (−) Hexobarbital und spektrale Anderungen des cytochrome P-450 in der Leber ascorbinsaurefrei ernahrter Meerschweinchen. Hoppe-Seyler's Z. Physiol. Chem. **354:** 238–242.
9. GINTER, E. 1973. Cholesterol: vitamin C controls its transformation to bile acids. Science **179:** 702–704.
10. DEGKWITZ, E. & K. S. KIM. 1973. Comparative studies on the influence of L-ascorbate, D-arabino-ascorbate and 5-OXO-D-gluconate on the amounts of cytochrome P-450 and b_5 in liver microsomes of guinea pigs. Hoppe-Seyler's Z. Physiol. Chem. **354:** 555–561.
11. KUENZIG, W. A. 1972. Ph.D. Thesis. New York University. New York, N.Y.
12. JONDORF, W. R., R. P. MAICKEL & B. B. BRODIE. 1958. Inability of newborn mice and guinea pigs to metabolize drugs. Biochem. Pharmacol. **1:** 352–354.
13. AVENIA, R. W. 1972. Ph.D. Thesis, Cornell University. Ithaca, N.Y.
14. VILTER, R. W. 1967. *In* The Vitamins. W. H. Sebrell, Jr. & R. S. Harris, Eds. **1:** 501. Academic Press. New York, N.Y.
15. SATO, P. H. & V. G. ZANNONI. 1974. Stimulation of drug metabolism by ascorbic acid in weanling guinea pigs. Biochem. Pharmacol. **23:** 3121–3128.
16. ZANNONI, V., M. LYNCH, S. GOLDSTEIN & P. SATO. 1974. A rapid micromethod for the determination of ascorbic acid in plasma and tissues. Biochem. Med. **11:** 41–48.

Discussion

Dr. I. B. Chatterjee: Did you measure the enzyme activity in the supernatant fraction to see if the enzymes are released? Your work indicates that it takes about 7–10 days for the reversal. That means that during this time, structural integrity is again gained.

Dr. V. Zannoni: We looked at the supernatant in terms of release, especially in the animals that were 3 weeks scorbutic. There was no indication of activity there. I agree that a structural change may occur. With prototype drugs, such as aniline, a type-II binder, we obtain a definite alteration in the binding spectrum that can be reversed with compounds like ascorbyl palmitate. So, a structural alteration may really occur, which then affects the metabolism. We're certainly interested in the phosphatidyl choline portion of the lipid portion of the microsomes. We looked at lipid peroxidation to see if it increased or decreased with deficiency, and it remained the same.

Dr. A. E. Kitabchi: In regard to the specificity of the organ, have you studied the steroid 21-hydroxylase in guinea pigs? Second, if induction is blocked by either actinomycin or cycloheximide, in bovine adrenal microsomes, ascorbic acid has no stimulating effects on the 21-hydroxylase system.

Dr. Zannoni: We didn't test the 21-hydroxylase. I think Dr. Avenia and Dr. Kamm studied steroid hydroxylation in deficiency. As I recall, they found a decrease in activity in steroid hydroxylation. We don't think that the increase we observed with increased vitamin C is due to induction in the sense of protein synthesis; rather, we think it's a protection or reduction of something on the membrane or protection against an alteration. We are not looking at this as phenobarbital induction. There is no evidence of this phenomenon. The vitamin-C-deficient animal can induce the same-fold increase as a normal animal in terms of protein synthesis.

Question: Do you believe that cytochrome *c* is an appropriate acceptor for the NADPH reductase system?

Dr. Zannoni: Cytochrome *c* is used because of its availability as an electron acceptor.

Dr. W. B. Smith: Have you tried other reducing agents?

Dr. Zannoni: Yes, we've used glutathione, the 2,6 dye, isoascorbate, and ascorbyl palmitate.