

METABOLIC INTERACTIONS BETWEEN L-ASCORBIC ACID AND DRUGS

A. H. Conney, George A. Bray, Carole Evans, J. J. Burns

Laboratory of Chemical Pharmacology, National Heart Institute, Public Health Service, Bethesda, Md.; New York University Research Service, Goldwater Memorial Hospital, Welfare Island, New York, N.Y.; The Wellcome Research Laboratories, Tuckahoe, N.Y.

Interrelationships between the metabolism of L-ascorbic acid and drugs have been the subject of numerous studies. Two aspects of this problem that will be considered here are as follows: (1) The ability of drugs to stimulate the synthesis and metabolism of ascorbic acid, and (2) the ability of ascorbic acid to influence the metabolism of drugs.

Effect of Drugs on Ascorbic Acid Metabolism

Drug-induced ascorbic acid synthesis. Various drugs possessing completely unrelated chemical and pharmacological properties have been shown to stimulate markedly the urinary excretion of L-ascorbic acid in rats.¹⁻³ They include the hypnotics: Chloretone and barbital; the analgesics: aminopyrine and antipyrine; the muscle relaxants: orphenadrine and meprobamate; the antirheumatics: phenylbutazone and oxyphenbutazone; the uricosuric agent, sulfipyrazone; the antihistaminics: diphenhydramine and chlorcyclizine; and the carcinogenic hydrocarbons: 3-methylcholanthrene and 3,4-benzpyrene.

Of considerable interest is the potent stimulatory effect on ascorbic acid synthesis exerted by the carcinogenic hydrocarbons 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, and 3,4-benzpyrene. The striking effect of a single 10-mg. intraperitoneal injection of 3-methylcholanthrene is shown in FIGURE 1. For comparison, the effect of a 40-mg. dose of Chloretone is also given. By 6 days after 3-methylcholanthrene administration, the urinary excretion of ascorbic acid was about 70 times greater than the control value and, in fact, during the 19-day period about 140 mg. of the vitamin was recovered in the urine. It will be noted that Chloretone within the first day exerts an effect on ascorbic acid excretion that reaches a peak by the third day and falls to a low value by the fifth day. In contrast, 3-methylcholanthrene exerts no effect for 2 days, but then urinary ascorbic acid excretion increases rapidly and remains elevated for over 18 days.

In another experiment rats were injected intraperitoneally with 10 mg. of the hydrocarbon daily for 3 to 5 days, and the urinary excretion was measured at various intervals thereafter. The urinary excretion of ascorbic acid increased from control values of about 0.3 mg. per day to values of 17 mg. per day by 6 days after the dose. Even by 50 days after administration of the hydrocarbon, the levels of ascorbic acid in urine were still markedly elevated over the control levels. No definite information is available at the present time on the prolonged effect of 3-methylcholanthrene on ascorbic acid synthesis. The possibility that the hydrocarbon remains in the animal by localization in fat depots is now under investigation.

Turnover rate of ascorbic acid. In order to show further the marked effect

of drugs on ascorbic acid synthesis the turnover of ascorbic acid was determined in rats treated with 3-methylcholanthrene, Chloretone, and pentobarbital; the data are given in FIGURE 2. In these experiments the drugs were given for several days, and then a tracer dose of L-ascorbic acid-1-C¹⁴ was administered intraperitoneally to control or drug-treated rats. The specific activity of ascorbic acid in daily urine samples was determined at various times after the dose of the labeled compound.⁴ In each case, 24 hours were allowed to lapse before the first specific activity value was determined in order to permit sufficient time for equilibration of the radioactive ascorbic acid with nonlabeled ascorbic acid in the animal. The specific activity values obtained have been

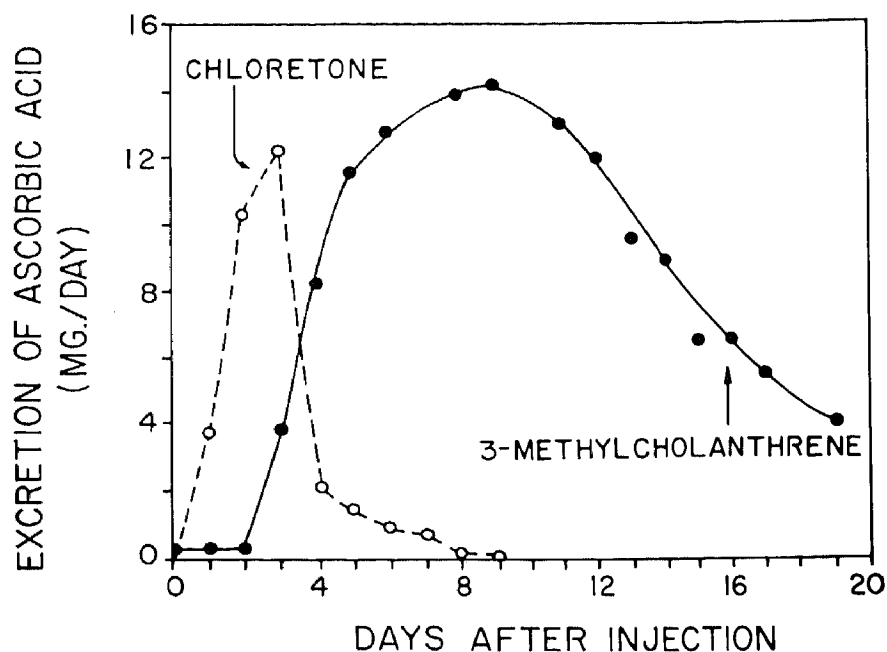


FIGURE 1. Urinary excretion of L-ascorbic acid by rats following a single intraperitoneal injection of 3-methylcholanthrene (10 mg.) or Chloretone (40 mg.).

plotted on a log scale at the mid-point of each urine collection period. The data show that 3-methylcholanthrene, Chloretone, and pentobarbital markedly shorten the half life of ascorbic acid in the rat. For example, in the normal rat the half life is 3 days; in the 3-methylcholanthrene-treated animal it is only 0.8 day.

From these data it is possible to calculate the body pool and turnover rate of ascorbic acid;⁴ these values are given in TABLE 1. In untreated rats, the body pool of ascorbic acid is 10.7 mg. per 100 gm. body weight, while in drug-treated animals the body pool of ascorbic acid about doubles. The turnover rate of ascorbic acid (the amount synthesized and metabolized or excreted each day) in control rats was about 2.6 mg. per 100 gm. rat per day. This value was increased about 10-fold by pretreating the rats with Chloretone or 3-

methylcholanthrene, and about 5-fold by pretreating the animals with pentobarbital.

The amount of ascorbic acid excreted daily in the urine did not account for all of the ascorbic acid synthesized each day. For example, in control rats about 1.8 mg. ascorbic acid per 100 gm. body weight was metabolized each day, whereas in the pentobarbital-, 3-methylcholanthrene-, or Chloretone-treated rats, this value increased to about 10 mg. per 100 gm. body weight per day

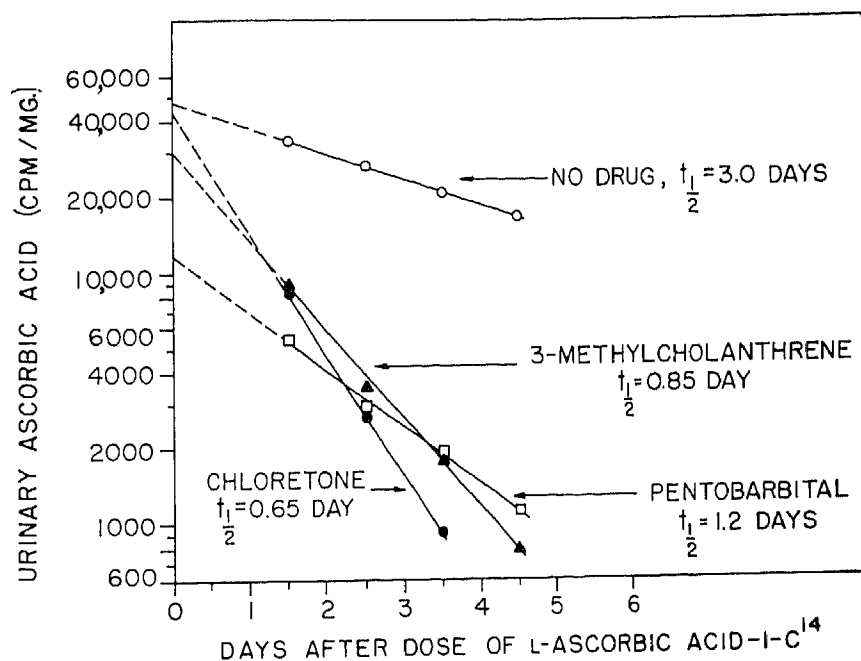


FIGURE 2. Turnover rate studies with L-ascorbic acid in drug-treated rats. Pentobarbital (30 mg.) or Chloretone (45 mg.) was administered orally for several days prior to administration of L-ascorbic acid-1-C¹⁴. 3-Methylcholanthrene (10 mg.) was injected intraperitoneally daily for 4 days; the L-ascorbic acid-1-C¹⁴ 5 days later.

TABLE 1
EFFECT OF DRUGS ON ASCORBIC ACID METABOLISM IN THE RAT*

Drug pretreatment	Body pool of AA (mg./100 gm.)	Turnover rate of AA (mg./100 gm./day)	Excretion of AA (mg./100 gm./day)	Metabolism of AA (mg./100 gm./day)
None	10.7	2.6	0.40	2.2
Chloretone	19.2	21.5	10.2	11.3
3-Methylcholanthrene	22.5	19.0	7.1	11.9
Pentobarbital	19.6	11.6	2.4	9.2

* Pentobarbital (30 mg.) or Chloretone (45 mg.) was administered orally for 4 to 7 days prior to the administration of L-ascorbic acid-1-C¹⁴. Methylcholanthrene (10 mg.) was injected intraperitoneally daily for 4 days, and the L-ascorbic acid-1-C¹⁴ was administered 5 days later. All values are given on a milligram per 100-gm. body weight basis.

(TABLE 1). Thus administration of drugs not only leads to increased urinary excretion of the vitamin, but also to a marked increase in its metabolic breakdown. Examination of the respiratory CO_2 at various intervals after administration of L-ascorbic acid-1- C^{14} showed that significant amounts of C^{14} were excreted by this route. In addition, evidence was found for the excretion of considerable amounts of labeled metabolites of ascorbic acid in the urine of drug-treated rats.

Agents inhibiting ascorbic acid synthesis. Administration to rats of adenosine triphosphate (ATP) has been reported to inhibit the synthesis of ascorbic acid.⁵ Experiments were carried out in our laboratory to investigate further this inhibitory effect of ATP. Three rats were given 25 mg. of Chloretone each by stomach tube daily for 7 days. In addition to this dosage of Chloretone, the animals then received by intramuscular injection 120 mg. of ATP daily, divided into 2 equal doses. The excretion of L-ascorbic acid dropped from an average value of 41 ± 2.0 mg./day, while the animals received only the Chloretone, to an average value of 15 ± 2.0 mg./day, when they received both Chloretone and ATP. Increasing the dosage of ATP to 150 mg./day, divided into 3 equal doses, did not decrease further the excretion of L-ascorbic acid.

The alkaloid lycorine has been reported to inhibit the synthesis of L-ascorbic acid in rats.⁶ In order to test this, we gave each of 3 rats 20 mg. of Chloretone daily by stomach tube for 3 days. The animals then received 6 mg. of lycorine, divided in two doses by subcutaneous injection, along with the dosage of Chloretone. L-Ascorbic acid excretion dropped from an average control value of 21.6 ± 3.0 mg. to 14.2 ± 1.7 mg. when the animals received both Chloretone and lycorine. The results indicate that lycorine had a distinct but far from complete effect in inhibiting L-ascorbic acid synthesis in Chloretone-treated rats.

Ascorbic acid synthesis via the glucuronic acid pathway. Administration of drugs to rats results in increased metabolism of glucose through the glucuronic acid pathway shown in FIGURE 3. The reactions of this pathway have been reviewed recently by Touster,⁷ Burns,⁸ Strominger,⁹ and Burns and Conney.¹⁰ According to this scheme, D-glucose is oxidized to D-glucuronic acid through the intermediate formation of uridine nucleotides. The D-glucuronic acid undergoes reduction to L-gulonic acid, which serves as the precursor of either L-ascorbic acid or L-xylulose.

Evidence that drugs stimulate the glucuronic acid pathway comes from the finding that administration of barbital or Chloretone to rats stimulates markedly the conversion of D-glucose-1- C^{14} or D-galactose-1- C^{14} to labeled D-glucuronic acid, L-gulonic acid, and L-ascorbic acid.^{3,11,12} This effect of barbital to stimulate glucose and galactose metabolism through the glucuronic acid pathway is shown in TABLE 2. Rats were given 100 mg. oral doses of barbital daily for several days and were then injected with D-glucose-1- C^{14} or D-galactose-1- C^{14} . Urine was collected for 24 hours, and the amount of radioactivity in isolated free D-glucuronic acid as well as in L-gulonic acid and L-ascorbic acid was determined. It is seen here that control rats convert less than 0.05 per cent of the D-glucose or D-galactose to D-glucuronic, L-gulonic, or L-ascorbic acids, and that considerable conversion took place in barbital-treated rats.

Additional experiments were carried out on the effect of 3-methylcholanthrene on the synthesis of L-ascorbic acid from D-galactose, and the results are shown in TABLE 3. In these experiments D-galactose-1-C¹⁴ was administered to rats, and the incorporation of C¹⁴ into urinary D-glucuronic, L-gulonic, and

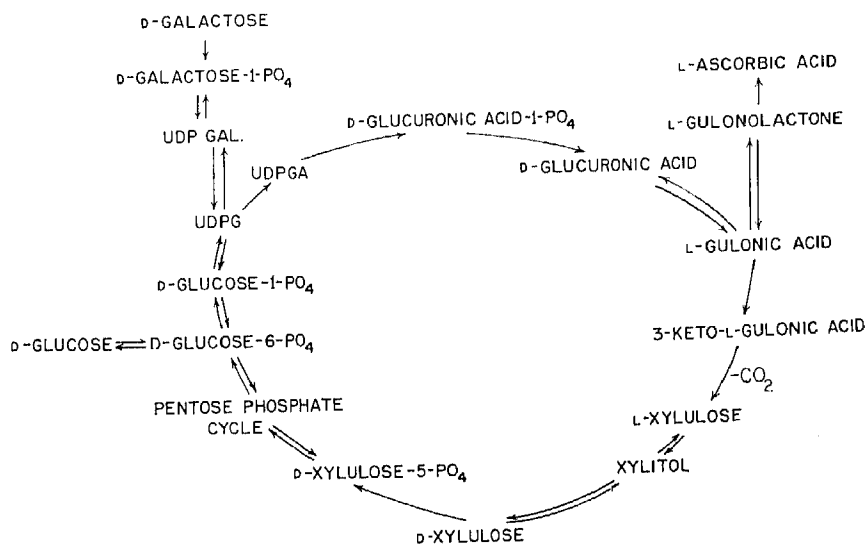


FIGURE 3. Glucuronic acid pathway.

TABLE 2
CONVERSION OF D-GLUCOSE-1-C¹⁴ AND D-GALACTOSE-1-C¹⁴ TO GLUCURONIC ACID PATHWAY INTERMEDIATES EXCRETED IN RAT URINE

Precursor	Per cent of administered C ¹⁴ in		
	D-Glucuronic acid	L-Gulonic acid	L-Ascorbic acid
<i>Control rats</i>			
D-Glucose-1-C ¹⁴	<0.03	<0.03	<0.02
D-Galactose-1-C ¹⁴	<0.05	<0.05	<0.05
<i>Barbital-treated rats*</i>			
D-Glucose-1-C ¹⁴	0.41	0.19	0.19
D-Galactose-1-C ¹⁴	1.49	0.65	0.77

* Rats were pretreated with 100 mg. of barbital orally for 7 days prior to the experiment.

L-ascorbic acids was measured. It will be noted that there was a considerable increase in the ability of 3-methylcholanthrene-treated rats to metabolize D-galactose-1-C¹⁴ to labeled L-gulonic and L-ascorbic acids beyond that observed in control rats. These data are similar to those obtained with barbital-treated animals (TABLE 2). However, in contrast to the extensive incorporation of D-galactose-1-C¹⁴ into D-glucuronic acid in barbital-treated rats, considerably

less was noted in the 3-methylcholanthrene-treated rats. Other studies have shown that barbital increased markedly the level of free D-glucuronic acid in urine, whereas little or no increased excretion was observed following 3-methylcholanthrene treatment. The possibility that 3-methylcholanthrene may stimulate the further metabolism of D-glucuronic acid as well as its formation is being investigated.

Evidence for the stimulatory effect of drugs on the glucuronic acid pathway (FIGURE 3) also comes from the earlier studies of Enklewitz and Lasker¹³ in human subjects with essential pentosuria. These subjects possess a metabolic defect that prevents the further metabolism of L-xylulose. When aminopyrine or antipyrine, drugs that stimulate ascorbic acid production, were administered to these subjects, the urinary excretion of L-xylulose was markedly elevated.

The exact mechanism by which drugs stimulate the formation of L-ascorbic acid via the glucuronic acid pathway is not known. The possibility that a renal mechanism is involved has been ruled out since drugs can stimulate ascorbic acid formation in nephrectomized rats.³ The formation of a glucuron-

TABLE 3
EFFECT OF 3-METHYLCHOLANTHRENE ON METABOLISM OF GALACTOSE-1-C¹⁴ TO
GLUCURONIC ACID PATHWAY INTERMEDIATES EXCRETED IN RAT URINE

Pretreatment	Per cent of administered galactose-1-C ¹⁴ in		
	D-Glucuronic acid	L-Gulonic acid	L-Ascorbic acid
None	<0.05	<0.05	<0.05
3-Methylcholanthrene*	0.14	0.61	0.56

* 10 mg. of 3-methylcholanthrene was injected intraperitoneally into rats daily for 4 days. Galactose-1-C¹⁴ was administered 5 days later.

ide by the drug is not required since barbital, a drug that stimulates markedly the production of free glucuronic acid and also L-gulonic and L-ascorbic acids, is excreted unchanged in the urine.¹¹ Several compounds such as borneol and α -naphthol, which are extensively conjugated as glucuronides, do not stimulate in rats the synthesis of L-ascorbic acid or its precursor L-gulonic acid.

In vitro studies. The possibility that pretreatment of rats with Chloretone may increase the activity of liver enzymes required for ascorbic acid synthesis was investigated. It was found that increased metabolism of D-galactose-1-C¹⁴ to free D-glucuronic acid occurred in liver homogenates derived from Chloretone-treated rats (TABLE 4). In this experiment, 300 to 325-gm. rats were given 40 mg. of Chloretone orally each day for 7 days. On the following day liver homogenates were prepared and incubated with D-galactose-1-C¹⁴ in the presence of adenosine triphosphate (ATP), uridine diphosphoglucose (UDPG), MgCl₂, diphosphopyridine nucleotide (DPN), and nicotinamide.¹² Formation of radioactive D-glucuronic acid was determined by a specific carrier dilution procedure. It is seen here that D-galactose is metabolized to D-glucuronic acid 3 to 4 times more readily by liver homogenates obtained from Chloretone-treated rats than by those from control rats.

More detailed studies on the effect of Chloretone on individual enzymatic

steps involved in ascorbic acid synthesis were then carried out. In these experiments 250- to 300-gm. rats were given 40 mg. of Chloretone orally daily for 7 days. They were killed on the following day, and the livers were used

TABLE 4
EFFECT OF CHLORETONE ON THE CONVERSION OF GALACTOSE-1-C¹⁴ TO D-GLUCURONIC ACID IN RAT LIVER HOMOGENATE

Rats	Per cent conversion to d-glucuronic acid
Control	0.21
Control	0.18
Chloretone*	0.68
Chloretone*	0.88
Chloretone*	0.77

* Rats were pretreated with 40 mg. of Chloretone orally for 7 days prior to the experiment.

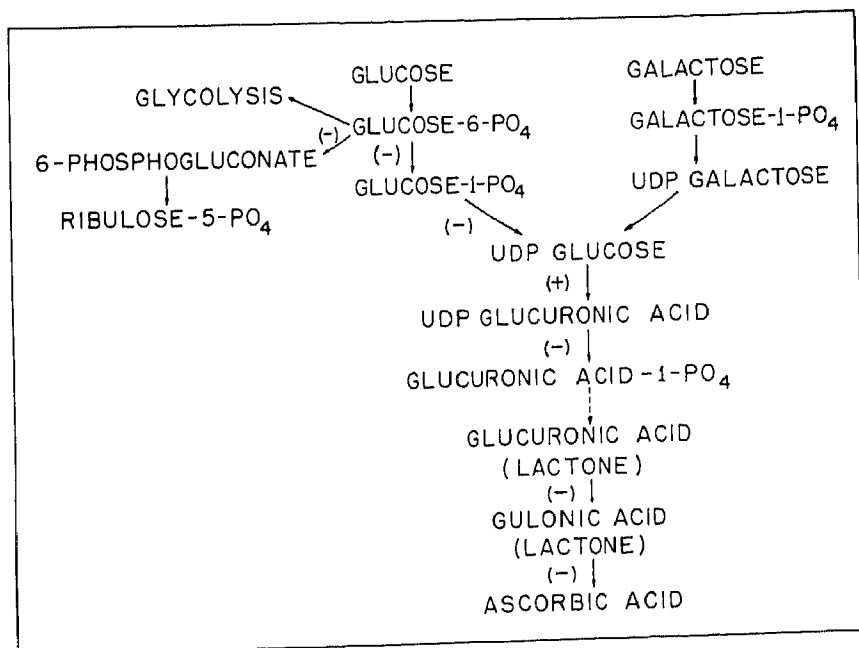


FIGURE 4. Effect of Chloretone pretreatment on liver enzymes involved in ascorbic acid biosynthesis. Chloretone (40 mg.) was administered orally for 7 days prior to the experiment.

to carry out studies on the various enzymatic reactions shown in FIGURE 4. The effect of Chloretone pretreatment on the activities of these liver enzymes is indicated by a plus or minus sign. Chloretone pretreatment did not stimulate the enzyme system that converts D-glucuronolactone to L-gulonolactone or the system that converts L-gulonolactone to L-ascorbic acid. In fact, the enzyme that converts L-gulonolactone to L-ascorbic acid was depressed about 50

per cent. These results are consistent with observations that drugs do not accelerate the *in vivo* conversion of D-glucuronolactone or L-gulonolactone to L-ascorbic acid.¹⁴ It therefore appears that drugs that stimulate ascorbic acid synthesis act at some step prior to D-glucuronic acid. The effects of Chloretone pretreatment on glycolytic enzymes, hexokinase, or glucose-6-phosphatase have not yet been investigated. The levels of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucomutase, and UDPG pyrophosphorylase were not significantly changed by Chloretone treatment. It was found, however, that the level of UDPG dehydrogenase, the system required to convert uridine diphosphoglucose to uridine diphosphoglucuronic acid (UDPGA), was elevated about 100 per cent in Chloretone-treated rats. The activity of the enzyme system in liver particulate that metabolizes UDPGA to D-glucuronic acid-1-phosphate was not stimulated. Only trace amounts of D-glucuronic acid were formed when UDPGA was incubated with livers from either control or Chloretone-treated rats.

TABLE 5
EFFECT OF PRETREATMENT OF RATS WITH CHLORETONE ON THE ACTIVITY
OF UDPG DEHYDROGENASE

Rats	Relative enzyme activity			
	Fresh	Aged*	Aged* with UDPG	Aged* with UDPGA
Control	100	0	33	77
Chloretone-treated†	185	125	—	168

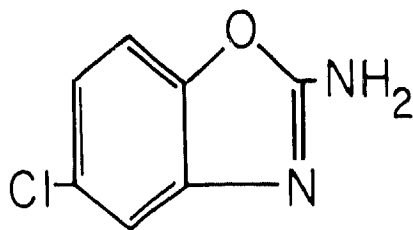
* The enzyme preparation was allowed to stand at 4° C. for 7 days.

† Rats were pretreated with 40 mg. of Chloretone orally for 7 days prior to the experiment.

Of the enzymes studied, only UDPG dehydrogenase levels were increased by Chloretone administration; therefore, this enzyme system was investigated in more detail. A number of the results are presented in TABLE 5. The interesting observation was made that upon standing at 4° C. the UDPG dehydrogenase activity in the high-speed liver supernatant fraction of control rats decreased in activity. Thus little or no UDPG dehydrogenase activity was observed in enzyme preparations that had been allowed to age at 4° C. for 5 to 7 days. On the other hand, similar enzyme preparations obtained from Chloretone-treated rats decreased only slightly in UDPG dehydrogenase activity when aged. This decrease in UDPG dehydrogenase with aging at 4° C. is prevented by allowing the enzyme to age in the presence of added UDPG, UDPGA, or uridine triphosphate (UTP), but not with added ATP or Chloretone. These studies raise the interesting possibility that Chloretone administration may accelerate *in vivo* the metabolism of UDPG to UDPGA by stabilizing UDPG dehydrogenase. This could possibly occur by increasing the levels of uridine nucleotides in liver. It is not known at present whether the effect of Chloretone in stimulating the synthesis of glucuronic acid may be explained by a stimulation and stabilization of UDPG dehydrogenase. Further studies are

in progress to determine the relevance of this enzymatic step in ascorbic acid synthesis.

Effect of drugs on drug-metabolizing enzymes. The results given here show that various drugs stimulate the metabolism of D-glucose and D-galactose to L-ascorbic acid through the glucuronic acid pathway. Recent studies indicate that drug administration exerts other biochemical effects. One effect studied in our laboratory is the ability of drugs to stimulate the activity of drug-metabolizing enzymes. It was found that drugs potent in stimulating ascorbic acid synthesis in rats are also potent in stimulating the activity of drug-metabolizing enzymes in liver microsomes.^{2,16} Examples of drugs that exert these two effects are 3,4-benzpyrene, 3-methylcholanthrene, Chloretone, phenobarbital, barbital, phenylbutazone, aminopyrine, orphenadrine, and chlorcyclizine. Examples of drug-metabolizing enzymes that are increased by administration of these inducer drugs include those enzymes that metabolize hexobarbital, pentobarbital, aminopyrine, phenylbutazone, 3,4-benzpyrene, and zoxazolamine.



ZOXAZOLAMINE

FIGURE 5.

Studies with zoxazolamine (structure shown in FIGURE 5) illustrate the effect of drugs on a drug-metabolizing enzyme. Zoxazolamine, a muscle-relaxant drug, is hydroxylated in the 6-position by liver microsomes to yield a pharmacologically inactive metabolite.¹⁶ In the experiment shown in FIGURE 6, 40-gm. rats were injected intraperitoneally with 75 mg./kg. of phenobarbital daily for 4 days or with 25 mg./kg. of 3,4-benzpyrene 24 hours before killing the animals and isolating their liver microsomes. It was found that pretreatment of the rats with these compounds markedly increased the activity of the liver microsomal enzyme system that metabolizes zoxazolamine. The increased enzyme activity was correlated with a shortened duration of zoxazolamine paralysis. The duration of action of zoxazolamine in control rats was 730 min., while in phenobarbital- or 3,4-benzpyrene-treated rats it was 102 min. or 17 min., respectively. Several lines of evidence indicate that these stimulators of enzyme activity actually induce the synthesis of drug-metabolizing enzymes.^{15,17,18} For instance, the increased activity of drug-metabolizing enzymes observed after pretreatment with phenobarbital or 3,4-benzpyrene can be completely blocked by administration of certain amino acid antagonists such as ethionine, an agent that inhibits protein synthesis. The effect of ethionine in blocking enzyme induction

can be completely overcome by the simultaneous administration of the amino acid methionine. In view of the effect of drugs in inducing the synthesis of drug-metabolizing enzymes, it is possible that drugs may also stimulate ascorbic acid synthesis by inducing the synthesis of enzymes of the glucuronic acid pathway. In this connection it is of interest to note that administration of ethionine to rats has been recently reported to block the ability of barbital and 3-methylcholanthrene to increase ascorbic acid synthesis.¹⁹

The mechanism by which drugs can both stimulate ascorbic acid biosynthesis and increase the activity of drug-metabolizing enzymes in liver microsomes is not known, but these effects appear to represent adaptive responses to drug administration that occur in the absence of the adrenal gland.^{11,17} We have

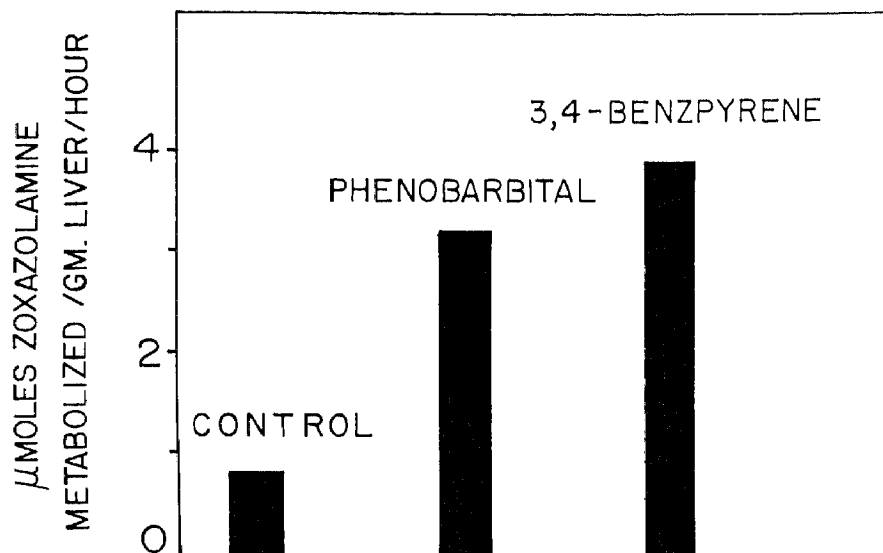


FIGURE 6. Stimulatory effect of phenobarbital and 3,4-benzpyrene administration on the activity of zoxazolamine hydroxylase in rat liver microsomes. Enzyme assays were carried out in the presence of a system that generated reduced triphosphopyridine nucleotide.¹⁵

been intrigued by the possibility that both of these responses to drugs may represent adaptations that are beneficial to the animal. Certainly, it is apparent that increased activity of drug-metabolizing enzymes in response to drug administration can result in accelerated drug detoxication. The effect of drugs to stimulate the glucuronic acid pathway (FIGURE 3) leads to increased production of UDPGA, D-glucuronic acid, and L-ascorbic acid. UDPGA is necessary for the conjugation of drugs as glucuronides. D-Glucuronic acid, when administered as its lactone, has been reported to protect animals from various toxic agents.²⁰ The possibility that L-ascorbic acid may be involved in the metabolism of drugs is pointed out in the following section.

Effect of Ascorbic Acid on Drug Metabolism

Reports have appeared in the literature indicating that vitamin C-deficient guinea pigs are unusually sensitive to various drugs. For example, Richards

and his co-workers reported that vitamin C-deficient guinea pigs were more sensitive than normal guinea pigs to pentobarbital and to procaine.^{21,22} In other studies Axelrod and his co-workers²³ have shown that the rate of *in vivo* hydroxylation of acetanilid and aniline was decreased in vitamin C-deficient guinea pigs.

Recent studies in our laboratory have indicated that vitamin C-deficient guinea pigs are more sensitive to the muscle-relaxant drug zoxazolamine than are normal guinea pigs. The increased sensitivity toward zoxazolamine can be explained by decreased activity of the enzyme system in liver microsomes that metabolizes this drug (TABLE 6). In these experiments the guinea pigs were placed on a scorbutogenic diet. One half of the animals were given daily doses of 10 mg. of ascorbic acid orally; the other half were given glucose. The animals were maintained on the scorbutogenic diet for 10 to 14 days, and at that time they showed no obvious signs of scurvy. It may be seen that the duration of zoxazolamine paralysis in guinea pigs receiving ascorbic acid supple-

TABLE 6
EFFECT OF ASCORBIC ACID DEFICIENCY IN GUINEA PIGS ON DURATION OF ZOXAZOLAMINE PARALYSIS AND ON THE ZOXAZOLAMINE-METABOLIZING ENZYME SYSTEM IN LIVER MICROSOMES*

Diet	Duration of zoxazolamine paralysis (min.)	<i>In vitro</i> metabolism (μg. zoxazolamine metabolized)
Ascorbic acid-supplemented†	156 ± 41 (10)	36 ± 12 (15)
Ascorbic acid deficient	309 ± 27 (20)	12 ± 8 (15)

* The duration of zoxazolamine paralysis was determined after the intraperitoneal injection of 100 mg./kg. of zoxazolamine. The *in vitro* enzyme assays were carried out by incubating liver microsomes from 375 mg. of liver with 100 μg. of zoxazolamine for 15 min. in the presence of a system that generated reduced triphosphopyridine nucleotide.¹⁵ The number of animals used are indicated in parentheses.

† The animals received 10 mg. of ascorbic acid orally each day.

ment was 156 min., whereas the guinea pigs that did not receive the vitamin were paralyzed for 309 min. The increased sensitivity of vitamin C-deficient guinea pigs was paralleled by decreased activity of the liver microsomal enzyme system that metabolizes zoxazolamine. Thus liver microsomes from ascorbic acid-supplemented guinea pigs metabolized an average of 36 μg. of zoxazolamine, while microsomes from the vitamin C-deficient animals metabolized an average of only 12 μg. of zoxazolamine. The addition of ascorbic acid *in vitro* to microsomes obtained from vitamin C-deficient guinea pigs did not increase the activity of this enzyme system. It should be emphasized that the decreased activity of the zoxazolamine-metabolizing enzyme system occurs at an early stage in vitamin C deficiency; this is observed before gross deficiency symptoms such as loss of weight and hair and severe joint manifestations are evident.

A model system consisting of L-ascorbic acid, ferrous ion, ethylenediamine-tetraacetic acid, and oxygen has been shown to catalyze the hydroxylation of such aromatic compounds as acetanilid, antipyrine, aniline, anthranilic acid, and kynurenine to yield products identical with those formed in the body.²⁴⁻²⁶ The importance of this system for the metabolism of drugs in the animal remains to be established.

Summary

The administration of various drugs to rats stimulates L-ascorbic acid synthesis from D-glucose and D-galactose through the glucuronic acid pathway. Increased metabolism of L-ascorbic acid also occurs in drug-treated rats. Although the enzymatic basis for increased ascorbic acid synthesis is not known, drugs appear to act on some step before the formation of D-glucuronic acid. The effect of Chloretone pretreatment to increase the activity of UDPG dehydrogenase in liver has been pointed out. It is of interest that drugs that stimulate ascorbic acid synthesis also induce the synthesis of drug-metabolizing enzymes in liver microsomes. It is possible that the effect of drugs on ascorbic acid synthesis also reflects induced enzyme synthesis.

Results presented here show that vitamin C deficiency makes guinea pigs more sensitive to the muscular-relaxant drug zoxazolamine by decreasing the activity of the liver microsomal enzyme system required for the metabolism of zoxazolamine.

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