cedes properdin in the reaction sequence [1] (Fig. 1). Thus, the initiating factor could be the analogue of C1, and the nephritic factor may be the activated form.

A third pathway has been described which is initiated by antibody mediated activation of C1 and which utilizes components of the previously described alternative pathway. This proposed third pathway by-passes the use of C4 and C2 to activate C3-9 [26].

The attack of C5 by the C5 convertases derived from either the classical or alternative pathways initiates the self-assembly process which, without further enzymic action, results in the production of the stable C5b-9 complex (thought to be composed of equimolar amounts of C5b, C6, C7, and C8 and six times as much C9) [1]. Theories proposing that the entire C5b-9 complex is either inserted into the membrane or that a small portion of the complex is extended through the membrane to allow exchange of intra- and extracellular constituents by means of internal hydrophilic channels have been put forward to explain the lysis of cells by complement [1,3].

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mental factors which can affect the extent of detoxification [6]. For example, the activity of the microsomal system is markedly increased with chronic exposure to a great variety of agents such as barbiturates, carcinogens and environmental chemicals [7]. This induction is a type of drug–drug interaction in which a drug can cause an increase in its own metabolism as well as the metabolism of other drugs. It is obvious that ascorbic acid could participate at a variety of levels in this complicated hepatic microsomal system, and a number of laboratories have contributed to the elucidation of the biochemical basis for the vitamin's participation [3–5,8–14].
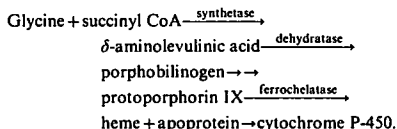
Vitamin C deficient diet

Adult guinea pigs maintained on a vitamin C deficient diet for 21 days had decreased overall drug metabolism, such as aniline hydroxylation, aminopyrine N-demethylation and p-nitroanisole O-demethylation, as well as decreased quantities of cytochrome P-450 and cytochrome P-450 reductase. The liver microsomal ascorbic acid was about 40% of normal; the animals had lost, at most, 5% of their body weight and frankly were not scorbutic [11]. In addition, weanling guinea pigs (90–110 g and 1–2 weeks of age) placed on a vitamin C deficient diet for a relatively short period (8–15 days) also showed a decrease in drug metabolism activity and, when given high supplements of vitamin C, showed substantial increases in overall drug metabolism activity and quantity of electron transport components (Table I). Furthermore, fetal guinea pig livers showed a marked variation in cytochrome P-450 and O-demethylase activity which correlated well with the concentration of liver ascorbic acid [15]. 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 livers with high ascorbic acid levels (above 17.0 mg/100 g liver) had much higher drug metabolism activity; cytochrome P-450 concentration was 84% of the dams while O-demethylase activity was 51% of the control dams.

Specificity studies with other reducing agents used in place of ascorbic acid in vivo, such as reduced 2,6-dichlorophenolindophenol dye, reduced glutathione, and D-isoascorbic acid, indicated that the vitamin is relatively specific in that these reducing agents did not significantly affect the levels of cytochrome P-450, NADPH cytochrome P-450 reductase or p-nitroanisole O-demethylation [15]. Reversals of decreased drug metabolism activities in vitamin C deficient animals by the in vivo administration of ascorbic acid showed that, although the quantity of liver ascor-

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Ascorbic acid and drug detoxification

V. G. Zannoni and L. E. Rikans

Ascorbic acid plays a part in maintaining adequate drug detoxification and its biochemical mode of action is of current interest.

It has been shown by both in vivo and in vitro experiments that vitamin C deficiency in guinea pigs results in decreased metabolism of a variety of drugs, steroids and cholesterol and that high supplements of the vitamin augment the activity of the drug enzyme system [1–5]. Our current knowledge of the liver microsomal pathway involved in the metabolism of many pharmacological agents is shown in Fig. 1. It involves an important heme protein,

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cytochrome P-450, which is reduced by NADPH via a flavoprotein, cytochrome P-450 reductase. For oxidative metabolic reactions the heme protein in its reduced state (Fe^{2+}) incorporates one atom of oxygen into the drug substrate and another into water. Many metabolic reductive reactions also utilize the hepatic cytochrome P-450 microsomal system. Associated with this important electron transport system is a lipid component, namely, phosphatidyl choline. Importantly, the hepatic drug metabolizing system is influenced by physiological and environ-

bic acid was restored to normal levels within three days, most of the drug enzyme activities required 6–10 days of ascorbic acid administration to reach normal levels [11]. Other studies using phenobarbital indicated that protein synthesis is operable in vitamin C deficiency in that overall drug oxidation activities (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 the extent of induction in normal animals.

Kinetic studies with *O*-demethylase indicate no significant difference in the apparent affinity of *p*-nitroanisole in normal $(K_m = 5.40 \times 10^{-4} \text{ M})$, ascorbic acid deficient $(K_m = 4.43 \times 10^{-4} \text{ M})$ or animals given high supplements of the vitamin $(K_m = 4.35 \times 10^{-4} \text{ M})$. In addition, no ·significant differences were found in K_m

Glycine + succinyl CoA $\xrightarrow{\text{synthetase}}$
δ-aminolevulinic acid $\xrightarrow{\text{dehydratase}}$
porphobilinogen $\rightarrow \rightarrow$
protoporphorin IX $\xrightarrow{\text{ferrochelatase}}$
heme + apoprotein→cytochrome P-450.

Experiments were carried out to determine if vitamin C deficiency affected the activity of δ-aminolevulinic acid synthetase, the rate-limiting enzyme in heme synthesis as well as δ-aminolevulinic acid dehydratase, the enzyme involved in the condensation of δ-aminolevulinic acid to porphobilinogen [18]. Synthetase activity in liver homogenates from weanling guinea pigs placed on a vitamin C deficient diet for 14–18 days did not differ significantly from the activity from control guinea pigs given daily supplements of vitamin C in their drinking water (1 mg/ml). In addition, there were no significant differences in the δ-aminolevulinic acid dehydratase activities.

It would be of interest to determine if there are any differences in ferrochelatase activity, since iron must be maintained in its ferrous state for incorporation into protoporphorin IX, and ascorbic acid could be involved at this level [19]. Furthermore, it will be of importance to examine degradation of cytochrome P-450, since a reduction of P-450 levels in ascorbic acid deficiency could also be due to an increased breakdown of the cytochrome.

Physical differences in microsomes

In contrast to the above results some differences in physical chemistry have been observed in microsomes prepared from deficient and normal animals. For example, cytochrome P-450 prepared from ascorbic acid deficient livers is less stable to sonication, dialysis, and treatment with metal chelators. The decrease in cytochrome P-450 and *O*-demethylase activity associated with dialysis could be prevented by the addition of ascorbic acid [14,16]. Results from several different kinds of studies indicate that ascorbic acid may be closely associated with cytochrome P-450. The molar ratio of microsomal ascorbic acid to cytochrome P-450 was found to be in the order of 1:1, and this ratio was maintained during ascorbic acid deficiency in liver and adrenal tissue, during dialysis, on storage, and during partial purification of the cytochrome. In addition, ascorbic acid protects cytochrome P-450 and aniline hydroxylase activity from inhibition by ferrous iron chelators such as α,α'-dipyridyl. The chelator reacts with cytochrome P-450 and prevents the formation of the reduced cytochrome P-450–CO spectrum. α,α'-Dipyridyl, in turn, gives a spectrum with the cytochrome at 450 nm, and ascorbic acid protects the cytochrome

TABLE I
The effect of in vivo administration of ascorbic acid on drug metabolism in weanling guinea pigs.

Treatment	Ascorbic acid	P-450	P-450 reductase	*p*-Nitroanisole *O*-demethylase	Aminopyrine *N*-demethylase
Chow (5)	27	0.068 ± 0.002	10.2 ± 0.7	10.5 ± 1.0	15.7 ± 0.4
Ascorbate (25 mg) (6)	8	0.060 ± 0.004	7.5 ± 0.8	8.0 ± 0.5	15.9 ± 2.3
Ascorbate (7 mg) (5)	6	0.060 ± 0.004	7.0 ± 0.8	7.5 ± 0.5	14.1 ± 0.9
Deficient diet (15-day) (6)	< 1.0	0.047 ± 0.002	3.4 ± 0.4	2.6 ± 0.2	10.0 ± 0.8

Weanling male albino guinea pigs, Hartley strain, were placed on various diet regimens as given in the table. Initial body weight of the animals ranged from 110 to 120 g. The body weights of animals on the ascorbic acid deficient diet did not differ significantly from the other groups. Groups of animals given the deficient diet were supplemented with either 25 or 7 mg of ascorbic acid given orally each day for 15 days. The number in parentheses equals number of animals in each group.

The concentration of ascorbic acid is given as mg/100 g liver; cytochrome P-450 equals μmoles/100 mg microsomal protein; cytochrome P-450 reductase activity equals μmoles P-450 reduced per hr per 100 mg microsomal protein at 27°; *O*-demethylase activity equals μmoles *p*-nitrophenol formed per hr per 100 mg microsomal protein at 27°; *N*-demethylase activity equals μmoles formaldehyde formed per hr per 100 mg microsomal protein at 27°. Values are given as the mean $\pm$S.E.

constants with aminopyrine *N*-demethylase in these groups [4]. Furthermore, the decrease in drug metabolism caused by ascorbic acid deficiency was not due to increased lipid peroxidation nor was there a significant qualitative or quantitative alteration in the important microsomal phospholipid component, phosphatidyl choline [14].

Impairment of heme synthesis

The possibility that the decreased quantity of cytochrome P-450 in ascorbic acid deficient microsomes may result from an impairment in heme synthesis was investigated. It was found that cytochrome P-450 levels returned to normal when scorbutic guinea pigs were injected with δ-aminolevulinic acid, a key substrate for heme biosynthesis [17]. A general scheme for the synthesis of heme is indicated below.
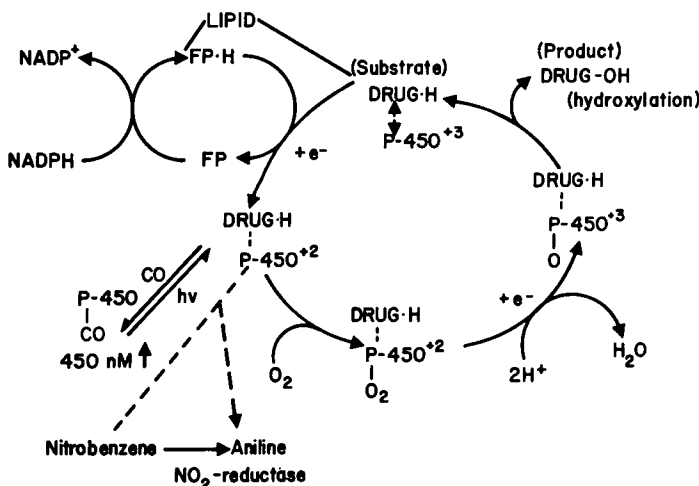

Fig. 1. Hepatic microsomal electron transport for drug oxidation and reduction.

from interaction with the chelator. The formation of a CO ligand with reduced cytochrome P-450 is the basis of determining the quantity of heme protein in the microsomes (Fig. 1). These studies suggest that there is an interaction between ascorbic acid and cytochrome P-450, involving the reduced form of the heme iron [14,16].

The need for vitamin C in the detoxication of drugs in humans may be important, especially during growth. In addition, the possibility should be considered that those individuals with insufficient intake of vitamin C may show sensitivity and possible toxicity to drugs. This would be true, for example, if increased formation of metabolites is necessary to ensure adequate detoxification. Of interest would be the time required for humans on a low intake of vitamin C to re-establish sufficient levels of drug metabolism after supplementation with the vitamin. In this respect increasing the intake of ascorbic acid in man above the recommended allowance (70–75 mg per day) to observe the effect on the biological plasma half-life of a variety of drugs as well as steroids and cholesterol may be of clinical importance.

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Brown fat mitochondria

David G. Nicholls

Brown fat mitochondria can short-circuit their respiratory chain to generate heat.

Our normal response to cold is to shiver. Shivering is caused by small muscular contractions which dissipate metabolic energy as heat and so help to restore our body temperature. There is, however, an alternative mens of generating heat under certain conditions which has been termed 'non-shivering thermogenesis', and which is important at birth, when an immense cold-shock must be overcome very rapidly, as well as being vital for the cold-adaptation of a variety of small mammals. Non-shivering thermogenesis is, in addition, an essential mechanism for hibernators, as heat can be generated even when the animal's body temperature is very low, and thus prevent the animal from literally freezing.

The most important site of non-shivering thermogenesis is in brown fat, a tissue which appears to be specialized solely for heat production. The tissue is perfectly adapted to its role. It can increase five-fold in weight on cold-adaptation, and has an ingenious blood supply which is directed towards warming the vital organs and which also involves a system of counter-current heating to ensure that blood entering the tissue is as warm as possible [1,2]. The cells contain many triglyceride droplets, and most of the remaining cytoplasm is packed with highly active mitochondria [2]. The tissue is controlled by the sympathetic nervous system which has noradrenalin-containing synapses in contact with the cells [2]. Release of the transmitter activates cyclic AMP production, which in turn activates a lipase, releasing free fatty acids as substrate for the mitochondria. The way in which brown fat mitochondria are adapted to handle this terminal oxidation is the subject of this article.

Physiological 'uncoupling'?

Mitochondria normally function to minimize the dissipation of chemical energy as heat, and one way that this is achieved is by a tight coupling of respiration to ATP production. For example, the high respiratory rate of exercising muscle is a consequence of the mitochondria attempting to restore the cytoplasmic ATP levels which were lowered by the ATPase action of the muscle. In brown fat, however, no such potent ATP-hydrolysing system can be found to account for the very high rates of respiration of which the tissue is capable [2–4]. If no way exists of hydrolysing ATP at a sufficient rate, then it follows that little ATP can be synthesized in the first place; in other words that brown fat mitochondria can uncouple the oxidation of fatty acids from the production of ATP. The isolated mitochondria have therefore been studied intensively in an attempt to localize the molecular nature of this 'uncoupling' (for reviews, see [2–4]).

The proton circuit

The chemiosmotic theory of Mitchell [5,6] provides a framework within which to investigate such physiological uncoupling. The proton circuit, the essence of the theory, is shown on p. 129. Substrate oxidation by the respiratory chain is linked to the expulsion of protons from the mitochondrial matrix, leading to the build-up of a proton electrochemical gradient (or protonmotive force) across the membrane which is analogous to the voltage developed by a battery, and is usually expressed in millivolts. Just as an electrical circuit requires a continuous pathway of electron conductors for current to flow, in the same way the proton circuit must be completed by some pathway that conducts protons back through the inner membrane. In most mitochondria the predominant pathway of proton re-entry is via the ATPase, the drop in potential of the protons being used to drive the energetically unfavourable synthesis of ATP.

Mitchell proposed [5,6] that not only was respiration tightly linked to the expulsion of protons, but also that proton re-entry was tightly linked to the production of ATP, so that when the cell had no further need for ATP synthesis, the proton current through the ATPase stopped, with the result that the proton electrochemical gradient built up, stopping further proton release (and hence respiration) by the respiratory chain. Clearly this automatic respiratory control could be overcome in two ways: if the link between respiration and proton extrusion could be broken, or if a way could be found of allowing pro-

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