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Vitamins and drug metabolism with particular reference to vitamin C

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Interaction between vitamins and drugs is influenced by the effects of drugs on the functions of vitamins, on the enzymes which they control, and the cell compartments which they maintain. However these aspects of their interaction take account only of the affinity and efficacy of the drugs and vitamins at their receptors. Another type of interaction depends on the ability of vitamins to influence drug metabolism. The converse of this is the ability of drugs to stimulate or inhibit the synthesis of vitamins. Drugs can influence synthesis of vitamins as a result of pharmacodynamic action in the cells. They can also affect bacterial synthesis of vitamins in the gastrointestinal tract. In this way oral administration of tetracycline produces deficiency of the B-complex by inhibiting its bacterial synthesis. By producing diarrhoea, drugs also alter absorption of vitamin K by affecting fat concentrations in the bowel contents, or by altering secretion of intestinal lipases.

The close relationship between vitamins and drugs is most directly exemplified by the deliberate modification of the chemical structure of members of the vitamin B complex so that their action as co-enzymes in energy-transfer mechanisms is competitively blocked. The chemical structure of folic acid was modified so as to produce methotrexate, a drug which competitively blocks folic acid and thus inhibits division of rapidly dividing leukaemic cells. In the same way co-trimoxazole was designed as a competitive blocker of bacterial metabolism by interfering with the conversion of *p*-aminobenzoic acid to folic acid, and with the conversion of folic acid to folinic acid. The interaction between vitamin C and drugs has been little studied, though it has been shown that tetracycline significantly reduces leucocyte ascorbic acid concentrations (Windsor, Hobbs, Treby & Astley Cowper, 1972), possibly by competitive blockage of intracellular metabolism (Goldsmith, 1956). As yet no competitive blockers of this vitamin have been produced. In view of the increased interest in vitamin C, its functions in the cell in relation to its interaction with drugs, and the mechanism by which it alters pharmacological effects, will be discussed.

Basic cellular mechanisms involved in the activity of ascorbic acid

Oxidation-reduction reactions of ascorbic acid. The effects of vitamin C can be attributed to the physico-chemical properties of the ascorbate system, ascorbic acid, and the dehydroascorbic entity. These are involved in oxidation-reduction activity, complex formation, hydrogen-bonding, and lowering of interfacial tension (Lewin, 1973). Ascorbic acid is a component of a reversible oxidation-reduction system in the cells in which it acts as a H receptor. A highly active intermediate is formed at an intermediate point (monodehydroascorbic acid), which is characterized

by a free-radical structure (Mason, 1965). This intermediate plays a direct and dominant role in the function of ascorbic acid as a cellular catalyst (Nutrition Reviews, 1957). A large number of compounds in living cells are reduced by ascorbic acid. Others are oxidized by the resultant dehydroascorbic acid. Ascorbic acid affects the ferritin mechanism, and promotes cellular absorption of iron, and haemoglobin formation. In virtue of its function as a H donor and receiver, ascorbic acid can act as a powerful buffering system in the cell. Such a system operates during transfer of Fe and dehydroascorbic acid through the erythrocytes, accompanied by the transfer of an electron, so that ascorbic acid enters the plasma (Loh & Wilson, 1970*a*, *b*). It then passes into the white blood cells in association with further energy transfer. Tissue concentrations of the vitamin should therefore be maintained within the saturated range in order to establish optimal tissue balance and health. In relation to other nutrients, ascorbic acid can produce a significant sparing effect on the vitamin B complex, including thiamin, riboflavin, folic acid and pantothenic acid, and on vitamins A and E (Terroine, 1962).

Formation of free ascorbate ion. Ascorbic acid exerts an indirect effect by the formation of free ascorbate ion. This is more readily oxidized than ascorbic acid itself. It is also inherently unstable in anaerobic conditions in comparison with the acid form. The indirect activity is exerted by way of various hormone receptor systems, with cyclic AMP and cyclic GMP acting as second messengers. Through the cyclic AMP system, ascorbate enhances the formation of adrenaline, which it protects from oxidative degradation. The adrenaline then potentiates adenyl cyclase, which activates formation of cyclic AMP from ATP. Ascorbate inhibits phosphodiesterase hydrolytic breakdown of cyclic AMP, and enhances the activity of cyclic GMP, which it also protects from hydrolytic degradation. Cyclic AMP and cyclic GMP potentiate numerous enzymic activities in the tissues which involve exocrine secretion, increased membrane permeability, hormone secretion and activity, as in the case of insulin, and interferon activation (Lewin, 1973).

The effects of drugs on synthesis of ascorbic acid

A large group of drugs which are unrelated pharmacologically or chemically induce the synthesis of ascorbic acid in the rat (Table 1). The rate of synthesis varies with the different drugs, the turnover being greater with the carcinogens than with the hypnotics. Drug administration not only leads to increased urinary excretion of the vitamin but also causes a significant increase in its metabolic breakdown by the microsomal enzyme system. Conney, Bray, Evans & Burns (1961) have demonstrated that enhanced synthesis occurs as a result of stimulation of the glucuronic acid pathway in rats. Following chloretone treatment, L-gulonic acid is changed by way of L-gulonolactone to L-ascorbic acid. Chloretone treatment of the rats, as a result of UDPG dehydrogenase (*EC* 1.1.1.22) activation, converts uridine diphosphoglucose to uridine diphosphoglucuronic acid from the precursor sugars glucose and galactose. It is possible that chloretone, and the other drugs causing ascorbic acid synthesis in rat liver, could operate by increasing the levels of uridine

nucleotides in the liver with resultant stimulation and stabilization of UDPG dehydrogenase.

Table 1. *Effects of drugs on ascorbic acid synthesis and liver microsome activity*

Hypnotics and anti-convulsants	Analgesics	Antihistamines
SM Chloretone	SM Aminopyrine	S Diphenhydramine
SM Barbital	SM Antipyrine	SM Chlorcyclizine
SM Pentobarbitone		S Mepyramine
SM Phenobarbitone		
M Diphenylhydantoin		
M Alcohol		
Muscle relaxants	**Anti-rheumatics**	**Carcinogens**
SM Orphenadrine	SM Phenylbutazone	SM Methylcholanthrene
S Meprobamate	SM Oxyphenylbutazone	SM 3,4 Benzopyrene
SM Zoxazolamine		
	Uricosurics	
	S Sulfinpyrazone	

S, Stimulation of ascorbic acid synthesis; M, Enhanced synthesis of drug-metabolizing enzymes.

Ascorbic acid and microsomal activity

Guinea-pigs become more sensitive to the muscle-relaxant drug zoxazolamine during ascorbic acid deficiency because the activity of the liver microsomal enzyme system required for its metabolism becomes decreased (Conney *et al.* 1961). Decreased activity of the zoxazolamine-metabolizing enzyme system occurs early in the development of scurvy, before gross deficiency symptoms such as alteration in the hair, loss of weight and changes in the joints become evident. Subsequently Kato, Takonaka & Oshima, (1969) have confirmed that the hydroxylating activity of liver microsomes on aniline, hexobarbitone, and zoxazolamine is significantly decreased in ascorbic acid deficiency, but that O-demethylation and N-demethylation activities of the microsomes were less affected. Ascorbic acid probably regulates biosynthesis of the hydroxylating enzymes or acts by stabilizing them. Deficiency of ascorbic acid is associated with reduced hepatic microsomal protein and various active enzymes (Chadwick, Crammar & Peoples, 1971). It has been shown that the concentrations of various members of the *P*-450 cytochrome enzyme complex are significantly reduced in scorbutic guinea-pigs when stimulated by phenobarbitone (Zannoni, Flynn & Lynch, 1972). The formation of glucuronic acid, which is involved in the metabolic formation of glucuronide, and of ascorbic acid, are closely inter-related. Altered supply of either therefore affects the other. A deficiency of ascorbic acid may therefore affect induction of glucuronide enzymes by lipid-soluble drugs.

Epileptic subjects treated with diphenylhydantoin (DPH) have significantly lower plasma and leucocyte ascorbic acid concentrations, and lower plasma folic

acid concentrations than normal subjects. They frequently have macrocytic anaemia which is responsive to folic acid supplementation. When DPH was administered to normal volunteers for 7 d, leucocyte ascorbic acid concentration became reduced on the 2nd day and did not return to normal until the 10th day after administration was started. Urinary excretion of ascorbic acid was significantly increased throughout the whole period, and plasma ascorbic acid concentration was reduced. The serum folic acid level did not change until the 9th day, when it was 60% of the original level. DPH is a potent inducer of hepatic drug-metabolizing enzymes. The low folic acid concentrations result from the increased catabolism of folic acid produced by the microsomal induction of enzymes arising from the DPH administration. Ascorbic acid is an essential metabolite for enzyme induction by DPH. Ascorbic acid synthesis is probably induced by DPH in animals possessing gulonolactone oxidase (*EC* 1.1.3.8), as it is by other drugs which increase production of hepatic microsomal enzymes. In man increased urinary excretion of ascorbic acid takes place in association with reduced plasma ascorbic acid concentrations when DPH gives rise to increased hepatic microsomal activity (Loh, 1973).

Ascorbic acid reduces the hepatic toxicity of hydrazine and other types of experimental hepatic damage (Beyer, 1943). Fatty degeneration of the hepatic cells is much more severe when hepatotoxic agents are administered during ascorbic acid deficiency. After anaesthesia with cyclopropane, ether or chloroform, plasma ascorbic acid rises with consequent increased urinary excretion of the vitamin, and returns to normal after 24 h (Beyer, Stutzman & Hafford, 1944). Scorbutic animals can be anaesthetized more rapidly, and recover more slowly, than healthy control animals receiving vitamin C supplements. By virtue of its effect on the hydroxylating mechanisms in the liver microsomes, supplementary vitamin C reduces the barbiturate sleeping time in rats (Richards, Kueter & Klatt, 1941).

Drugs acting on the central nervous system

The function of ascorbic acid in the brain is unknown, although it may be involved in olfaction (Ash, 1969), taste (Loh & Wilson, 1973) and in the production of anorexia arising from fenfluramine administration (Odumosu & Wilson, 1973*a*). Clonic and tonic convulsions are produced in guinea-pigs by administration of leptazol. The severity of the convulsions is inversely correlated with the concentration of brain ascorbic acid, which is most reduced in the mid-brain where it is probably being catabolized (Odumosu & Wilson, 1974). Administration of ascorbic acid preceding induction of the leptazol-induced convulsions reduces their severity. Yegnanarayan, Sarat & Joglekar (1973) have also demonstrated that leptazol- and strychnine-induced convulsions in mice are significantly reduced by prior administration of ascorbic acid, but that the vitamin does not affect the incidence of convulsions induced by methylamphetamine or caffeine. It was concluded by Odumosu & Wilson (1974) that ascorbic acid may play a major metabolic role in the brain. This is possibly related to the associated alterations in catecholamine content of the brain when the concentrations of ascorbic acid are altered (Izquierdo, Jofré & Acevedo, 1968).

Drugs affecting fat and carbohydrate metabolism

The anti-obesity action of fenfluramine in guinea-pigs is opposed by the administration of two parts (by weight) of ascorbic acid to one of fenfluramine. The anti-obesity effect of fenfluramine is associated with a significant reduction in hepatic ascorbic acid concentrations, and ascorbic acid catabolism is enhanced. When ascorbic acid deficiency exists in fenfluramine-treated guinea-pigs, hepatic and plasma cholesterol levels are also significantly reduced. However, when supplementary ascorbic acid is administered with the fenfluramine, the hepatic cholesterol is raised in comparison with values in normal guinea-pigs (Odumosu & Wilson, 1973*b*). Initial evidence indicates that fenfluramine exerts similar effects on ascorbic acid metabolism in humans (Wilson, 1974*a*). It is known that ascorbic acid may be involved in the production of insulin through the oxidation of sulphydryl groups occurring as a result of glutathione-ascorbic acid interaction. Fenfluramine is known to have a hypoglycemic effect (Turtle & Burgess, 1973) which occurs without the intervention of any insulin activity. The reported hypoglycemic effects of ascorbic acid are probably attributable not only to its action in promoting synthesis of insulin, but also to an indirect effect on the AMP-GMP mechanism affecting the uptake of glucose into muscle, in which fenfluramine also plays a role (Duhault & Boulanger, 1966; Butterfield & Whichelow, 1968).

Ascorbic acid and lysosomes

Ascorbic acid is involved in the structural and numerical control of lysosomes in guinea-pigs, and in the regulation of the activity of their enzymes (Desai, Sawant & Tappel, 1964; Lippi, Pulido & Guidi, 1966; Terroine & Hitier, 1969). Deficiency of ascorbic acid results in increased activity of lysosomal acid phosphatase in skeletal muscle. Stimulation of this enzymic activity is apparent from the 15th day of the scorbutogenic diet, when severe structural changes appear in the skeletal muscle (Hitier, 1968), and when weight loss commences (Odumosu & Wilson, 1973*c*). The enzymatic effects in the muscle do not differ appreciably from those found in human vitamin C deficiency (Mason, 1969). They probably result from labilization of the membranes of the lysosomes present in the muscle fibre. Subsequently the fibres are invaded by macrophages from the blood which give rise to acid hydrolases, amylsulphatase ribonuclease, cathepsin, β-galactosidase (*EC* 3.2.1.23) and β-glucuronidase (*EC* 3.2.1.31). This may represent an attempt at regeneration in the degenerating tissue, because a periodic increase in lysosomal enzyme activity occurs in tissue undergoing active regeneration. This coincides with the cycle of mitotic division (Adams, 1963).

Briggs & Briggs (1973) have reported that oestrogen-progestogen oral contraceptives reduce leucocyte and plasma ascorbic acid concentrations in healthy women. Hypovitaminosis C leads to large-scale labilization of testicular lysosomes in the young scorbutic guinea-pig. Administration of serum gonadotrophin to scorbutic guinea-pigs prevents this labilization, but does not restore the normal histological appearance of the testes (Hitier, 1970). In contrast to this autophagy produced by deficiency of

vitamin C, riboflavin and pyridoxine deficiencies prevent normal formation of interstitial gland and seminiferous tubule formation. It is clear that deficiency of vitamin C is associated with reduced reproductive capacity in both sexes. Deficiency during pregnancy is associated with increased incidence of congenital abnormalities (Nelson & Forfar, 1971). It can be concluded that drugs which predispose to reduced ascorbic acid concentrations during the reproductive period not only reduce fertility, but also have teratogenic potentiality.

Other abnormalities in cell metabolism may develop during deficiency of vitamin C. These are associated with an increase in deoxyribonuclease (Hitier, 1967) and cathepsin (Moruzumi, 1960) activity in the liver and spleen. Lysosomal activity may be responsible for the disturbances in hepatic metabolism and antibody production which are associated with reduced ascorbic acid levels during disease (Wilson, 1974*a*). Collagenase (clostridiopeptidase A; *EC* 3.4.4.19) has been identified in the lysosomes by Wynn (1967). It is activated during scurvy in guinea-pigs (Barnes & Kodicek, 1972) and it has been suggested that reduction in tissue ascorbic acid brings about desaturation of the mucopolysaccharides through release of hyaluronidase, β-glucuronidase and β-galactosidase from the lysosomes. The activation and release of collagenase leads to catabolism and break-down of connective tissue (Woessner, 1965). This can result from tissue desaturation of ascorbic acid during disease (Wilson, 1974*a*).

In its capacity as a biological oxidant, ascorbic acid inhibits lipid peroxidation. It thereby prevents the liberation of free acids, and thus plays an important role in fat metabolism. Its reducing properties are therefore largely responsible for main-

Table 2. *Drugs, hormones and other agents which promote the labilization and stabilization of lysosomes*

Labilization	
In vitro	In vivo
Digitonin	Vitamin A
Progesterone and Testosterone	Thyrotropin
Diethylstilbestrol	Endotoxins
Deoxycorticosterone	Antigen-antibody reactions
Etiocholanone	Phosphase of mitosis
Cantharidin	Metamorphosis and tissue resorption
Cysteine	Virus infections
Vitamin A	
X-irradiation	
Ultra-violet irradiation	

Stabilization	
In vitro	In vivo
Cortisol	Cortisone and analogues
Prednisone	Chloroquine
Beta-methasone	Serum factors in autoimmunity
Cholesterol	Tolerance to endotoxin
Chloroquine	? Aspirin
Antihistamines	? Alcohol
Serum factors in autoimmunity	

taining the structural and functional integrity of the lysosomes. It also induces oxidation of thiol groups in human plasma proteins (Meacham, 1968). Since the membranes bounding lysosomes resemble the unit membranes which surround erythrocytes, a number of membrane-active drugs, vitamins, toxins and other substances can act to labilize or stabilize the surface of the lysosomes (Table 2). Most of the labilizing agents are also haemolytic, and may affect mitochondria as well. Among the stabilizing agents, the anti-inflammatory steroids cortisone, cortisol, prednisone, prednisolone and beta-methasone act both in vitro and in vivo in the living cell. They stabilize the lysosomes against excess vitamin A, streptolysin O, progesterone and carbon tetrachloride. Anti-inflammatory steroids exert their action at least in part upon the membranes of lysosomes. They significantly reduce the pyrogenic and inflammatory effects of u.v. irradiation, and antigen-antibody reactions, as does chloroquine. Cholesterol protects lysosomes against rupture at acid pH and some anti-histamines also stabilize lysosomes. Mepyramine has been shown to inhibit the Schwarzmann reaction when combined with administration of vitamin C, and aspirin alters ascorbic acid metabolism in health (Loh, Watters & Wilson, 1974), and in disease (Wilson, 1974*b*).

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