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Vitamin Neurotoxicity

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Contents

Abstract

Vitamins contain reactive functional groups necessary to their established roles as coenzymes and reducing agents. Their reactive potential may produce injury if vitamin concentration, distribution, or metabolism is altered. However, identification of vitamin toxicity has been difficult. The only well-established human vitamin neurotoxic effects are those due to hypervitaminosis A (pseudotumor cerebri) and pyridoxine (sensory neuropathy). In each case, the neurological effects of vitamin deficiency and vitamin excess are similar. Closely related to the neurological symptoms of hypervitaminosis A are symptoms including headache, pseudotumor cerebri, and embryotoxic effects reported in patients given vitamin A analogs or retinoids. Most tissues contain retinoic acid (RA) and vitamin D receptors, members of a steroid receptor superfamily known to regulate development and gene expression. Vitamin D_3 effects on central nervous system (CNS) gene expression are predictable, in addition to the indirect effects owing to its influence on calcium and phosphorus homeostasis. Folates and thi-

amine cause seizures and excitation when administered in high dosage directly into the brain or cerebrospinal fluid (CSF) of experimental animals but have rarely been reported to cause human neurotoxicity, although fatal reactions to iv thiamine are well known. Ascorbic acid influences CNS function after peripheral administration and influences brain cell differentiation and 2-deoxyglucose accumulation by cultured glial cells. Biotin influences gene expression in animals that are not vitamin-deficient and alters astrocyte glucose utilization.

The multiple enzymes and binding proteins involved in regeneration of retinal vitamin A illustrate the complexity of vitamin processing in the body. Vitamin A toxicity is also a good general model of vitamin neurotoxicity, because it shows the importance of the ratio of vitamin and vitamin-binding proteins in producing vitamin toxicity and of CNS permeability barriers. Because vitamin A and analogs enter the CNS better than most vitamins, and because retinoids have many effects on enzyme activity and gene expression, Vitamin A neurotoxicity is more likely than that of most, perhaps all other vitamins. Megadose vitamin therapy may cause injury that is confused with disease symptoms. High vitamin intake is more hazardous to peripheral organs than to the nervous system, because CNS vitamin entry is restricted. Vitamin administration into the brain or CSF, recommended in certain disease states, is hazardous and best avoided. The lack of controlled trials prevents us from defining the lowest human neurotoxic dose of any vitamin. Large differences in individual susceptibility to vitamin neurotoxicity probably exist, and ordinary vitamin doses may harm occasional patients with genetic disorders. Several vitamins, including A, D_3, ascorbate, biotin, folates, pyridoxine, and thiamine have been found to have noncoenzyme effects that indicate neurotoxic potential. Increasing numbers of vitamins are being reported to alter gene expression by noncoenzyme mechanisms. Data are presented showing that several vitamins (biotin, folic acid, pyridoxal, and RA) increase ^{45}Ca influx in cultured neural cells. These effects on calcium permeability correlate with neurotoxic potential.

Index Entries: Vitamins; vitamin A; ascorbic acid; blood-brain barrier; intrathecal drug administration; neurotoxicity; calcium; pyridoxine.

Introduction and Historical Review

The 13 established vitamins are listed in Table 1, together with the date their structure was established, information about their CNS transport, and the presence of vitamin-binding proteins. Since all vitamins and many other drugs bind to serum albumin to some extent, it is not considered to be a vitamin-binding protein. The table suggests that all water-soluble vitamins have specific transport mechanisms and that most fat-soluble vitamins do not. However, few studies have examined the transport of fat-soluble vitamins other than across the intestinal mucosa. Studies of vitamin transport may be complex. One study of vitamin A and D entry into brain from blood suggests very poor penetration through the blood-brain barrier (Pardridge et al., 1985). However, another and probably sounder study indicates good CNS entry of several vitamin A analogs (Kalin et al., 1982). Very low CSF tocopherol levels also suggest restricted vitamin E entry into the CNS (Vatassery et al., 1991). Specific transport mechanisms for cellular uptake of water-soluble vitamins are likely to be found in all tissues because of the barrier to their passage across the lipid plasma membrane. All the coenzymes listed in Table 2, with the possible exception of vitamins E and K, are essential to the metabolism of nervous tissue. Brain is one of the few tissues apparently lacking vitamin K-dependent carboxylase (Vermeer, 1990), and it appears that neurons and glia grow well in media lacking vitamin E and in some cases, lacking either ascorbate or vitamin E.

Studies of cultured neurons and astrocytes in defined media (media lacking serum, embryo extracts and other ill-defined biological extracts), moreover, demonstrate that short-term survival of neurons and glial cells is possible in media containing as few as eight vitamins. The survival of neurons dissociated from chick sensory and sympathetic ganglia over 30 d was not reduced by omission of all supplementary vitamins

Table 1
Water-Soluble Vitamins

Vitamin	Structure established	Specific-binding proteins	CNS transport
Ascorbic acid (vitamin C)	1933	no	yes
Biotin	1941	yes	yes
Folates	1945	yes	yes
Niacin	1914	no	?
Pantothenic acid	1940	?	yes
Pyridoxine	1938	no	yes
Riboflavin	1935	yes	yes
Thiamine	1936	no	yes
Vitamin B12	1957	yes	yes
Fat-Soluble Vitamins			
Vitamin A	1931	yes	—
Vitamin D	1948	yes	yes
Vitamin E	1935	no	no
Vitamin K	1940	no	no

Table 2
Coenzymes Derived from Vitamins

Enzymatic transfer of hydrogen atoms	Vitamin precursor	Example
Nicotinamide adenine dinucleotide (NAD)	—	—
Nicotinamide adenine dinucleotide phosphate (NADP)	niacin	dehydrogenases
Flavin adenine mononucleotide (FAD)	riboflavin	mitochondrial flavoproteins
Coenzyme Q	vitamin E	
Enzymatic Transfer of other Molecules		
Coenzyme A- SH	pantothenic acid	choline acetyltransferase
Pyridoxal phosphate (PALP)	pyridoxine	transaminases, decarboxylases
Thiamine diphosphate	thiamine	transfers an activated aldehyde group
Tetrahydrofolic acid	various folates	one carbon metabolism,
Biotin	biotin	carboxylases
Adenosyl and methylcobalamin	vitamin B12	one carbon metabolism
Other vitamins		
Ascorbic acid: donor of reducing equivalents, antioxidant function		
Vitamin K: donates reducing equivalents in conversion peptide bound glutamate to -carboxyglutamate		
Vitamin D_3: activate nuclear receptors and calcium/phosphorus transport		

(Wakade et al., 1982). Ham's F-12 medium, used by Wakade et al., contains biotin, calcium pantothenate, folic acid, nicotinamide, pyridoxal and pyridoxine, riboflavin, thiamin, and vitamin B12; ascorbate, tocopherol, and fat-soluble vitamins are lacking. Neurons do not usually divide in culture, but proliferation and differentiation of human and rat glial cells proceeded satisfactorily in Ham's F-12 or Dulbecco's modified Eagle's medium (DMEM) (which contains only six of the vitamins found in the F-12 medium, albeit at somewhat higher concentrations than in

the F-12 medium). Biotin, not found in DMEM, was added, but the author's data indicate that it made little difference. Transferrin, selenium, and fibronectin were essential (Michler-Stuke and Bottenstein, 1982). Insulin and transferrin stimulate the survival of almost all cultured cells (in the absence of serum), but extra vitamins are required for only a few and then often only biotin.

As is true of transport studies, the need for water-soluble vitamins by cultured cells has been studied much more extensively than the need for fat-soluble vitamins. However, long-standing deficiencies of most vitamins impair the growth and functioning of the nervous system in vivo. Tissue culture systems select for robust cells that are maintained for periods of only a few weeks and fail to express the most complex network functions of the CNS. The nutritional requirements of tissue culture systems are less than in the in vivo situation, particularly the situation of embryos or infants in which neuronal division, migration, and differentiation are so prominent.

Pauling and others have postulated that some forms of mental and neurological illness may be vitamin-responsive; there is a popular belief or myth that increased vitamin intake may make healthy people stronger and cannot be harmful. Related to this belief is the hope that vitamin supplementation may improve intelligence, most often suggested for children and occasionally, adults as well. A few studies have compared intelligence test performance in children receiving vitamin and mineral supplements to controls. Results have been inconsistent, with some studies reporting small, statistically significant improvement in nonverbal test performance in children receiving the supplement (Editorial, 1991).

A recent study of California children aged 12–16 reported small but statistically significant gains in performance on nonverbal portions of the WISC-R in children receiving a complex vitamin and mineral supplement designed to provide 100% of the recommended daily allowances (RDA) (Schoenthaler et al., 1991). The groups were identical on many other test measures, and other children receiving 50 or 200% of the RDA did not differ from controls in test performance. The lack of benefit in children supplemented at the 50 and 200% levels suggests either that there is a narrow "therapeutic dose window" for vitamin benefit or that the results are statistical artifacts.

Advocates of the "vitamin effect" suggest that a subgroup of malnourished children respond to supplementation with improved nonverbal test performance. We cannot explain the selectivity of this effect for nonverbal test items, and the commercial gain from vitamin sales (Schoenthaler, for example introduced a commercial vitamin and mineral preparation after announcing his results) suggests the need for careful scrutiny. Because a small improvement in the intelligence of 10 or 20% of underprivileged children would be important, we need further and more sophisticated replications of this experiment. If the effect is genuine and attributable to malnutrition, we should also expect to find other characteristics that distinguish "vitamin/mineral responders" from the majority of children. Judging from the contentious letter of H. C. Eysenck (1991), readers should scrutinize the claims made with great care. Eysenck said that it was "breathtakingly naive" for a reviewer to suggest that some children suffer a decline in IQ from use of vitamin supplements. The thrust of this article is that such suspicions are warranted and that Eysenck's assumption that vitamins can do good but never harm is the naive one.

Patients with various inborn errors of metabolism, such as mitochondrial encephalomyopathies, have often been treated with multivitamin "cocktails" on the assumption that they may have a vitamin-responsive condition and that treatment will not hurt even if it does not help the patient (Forfar and Arneil, 1984). Beginning with pyridoxine-dependent seizures in 1954, more than 20 inborn errors of metabolism have been reported to be vitamin-responsive (Rudman and Williams, 1983).

Most of these diseases are very rare, and their response to vitamins is partial or transient (Scriver, 1985). Responses to supraphysiological vitamin intake in these hereditary diseases have been explained by reduced affinity of mutant enzymes for cofactors (Chuang et al., 1982), although mutations involving vitamin absorption from the gut, transport within the body, vitamin metabolism, and binding proteins might also produce increased vitamin requirements (Kim and Rosenberg, 1974). It is equally likely, however, that some mutations, diseases, and drugs may reduce vitamin tolerance. In recent years, common complaints, such as carpal tunnel syndrome, the so-called premenstrual syndrome, and common skin disorders, have been treated with high doses of vitamins (Williams et al., 1985) or vitamin analogs (Leyden, 1988). Since omission of vitamin B6 from infant formulas was associated with increased incidence of seizures (Bernstein et al., 1989), some physicians have treated childhood epilepsy of unknown cause with high doses of pyridoxine. Similar reasoning has suggested to others that psychotic illnesses, known to be associated with pellagra, might respond to megadoses of niacin.

General Models of Vitamin Function

Vitamin Coenzymes and Reducing Agents

Most vitamins exist in multiple forms, are associated with binding proteins, and are concentrated in tissues by specific transport processes. Vitamins are often essential cofactors for enzymatic reactions. This coenzyme function implies chemical reactivity because the coenzyme donates or receives a functional group or atom, requiring regeneration by a different enzymatic reaction for reuse. Table 2 presents a list of vitamin-dependent coenzymes. Many common enzymatic reactions such as decarboxylation, dehydrogenation, and transamination depend on vitamin-derived cofactors such as NAD or PALP (PALP). Other vitamins function as reducing agents or antioxidants without classical catalytic roles. This is discussed further in the section about ascorbate. Most vitamins require biotransformation to produce active coenzymes. Table 2 shows that only ascorbate, biotin, and vitamin E are metabolically effective without metabolic transformation. Even these vitamins require regeneration, however, to restore their active form after use. So-called vitamin K antagonists prevent recycling of the vitamin, for example (Suttie, 1985).

Figure 1 presents summary diagrams of simple and complex theories of vitamin function. In Fig. 1A, the vitamin is ingested, absorbed, and passively distributed to all tissues in the body, where it binds only to enzymes that use it as cofactor. Figure 1B presents a more realistic picture, using pyridoxine as an example, and indicates the presence of specific CSF transport processes, specific enzymes for generation of active coenzyme, including pyridoxal kinase, and the ability of B6 vitamers to nonspecifically pyridoxylate many proteins, often changing their properties.

Figure 1C summarizes the even more complex coenzyme role of vitamin A. This vitamin is required for regeneration of rhodopsin bleached by light in retinal rods. However, it must go to the retinal pigment epithelium (RPE) for regeneration, after which it returns to the rod outer segments (Okajima et al., 1990). Ocular vitamin A occurs as the 11-*cis* and all-*trans* isomers of retinol and its esters (Bridges, 1976). Light adaptation is associated with isomerization of retinol from the *cis* to the *trans* form, as rhodopsin is bleached. Now the rod outer segments must regenerate rhodopsin from a store of vitamin A that is in the wrong form and place (in mammals, only the pigment epithelium has the isomerase enzyme needed to produce *cis*-retinol; Fulton and Rando, 1987). About 80% of the now *trans*-retinol leaves retina (as a complex with the interphotoreceptor retinol binding protein (IRBP), and enters the RPE. Figure 1C provides the details of

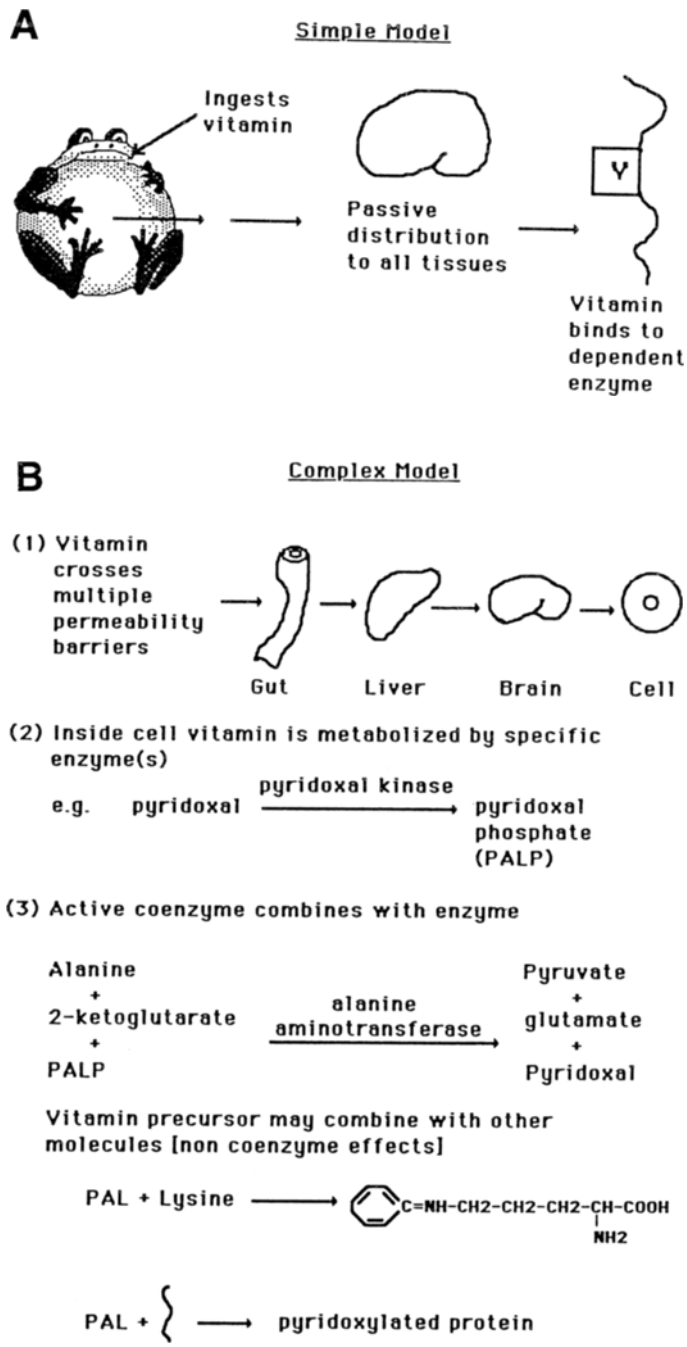
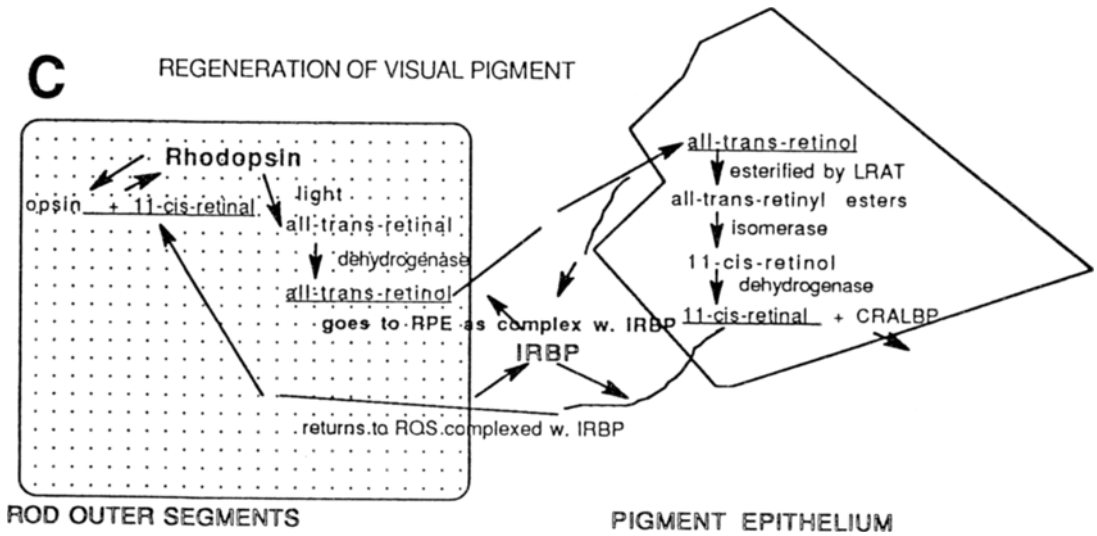
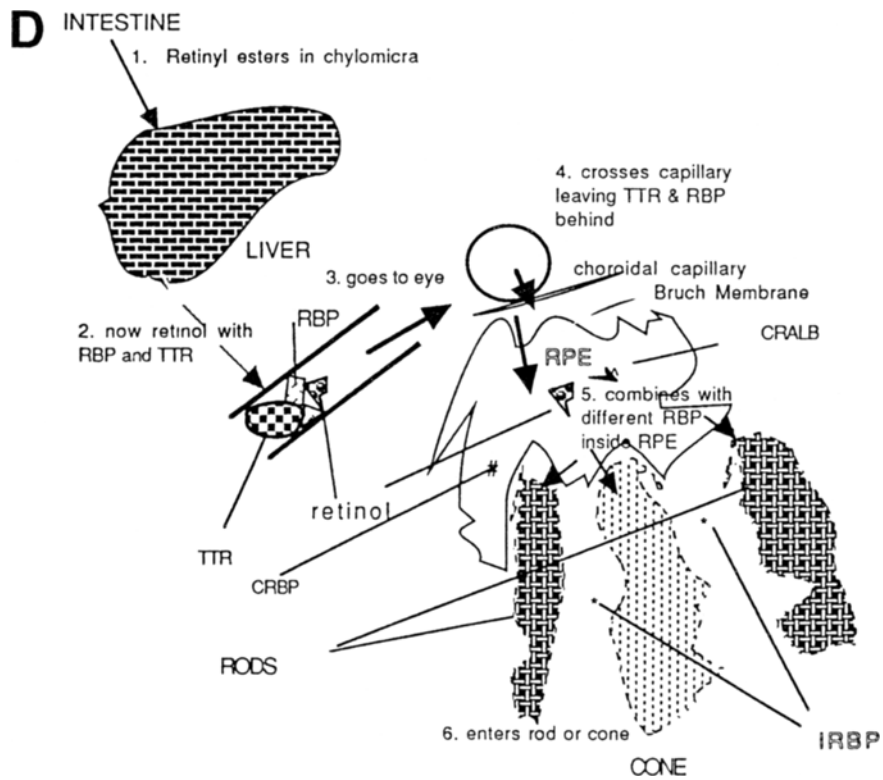


Fig. 1a. The simplest model of vitamin distribution. The vitamin goes to all tissues and binds only to enzymes that use it as cofactor (**A**). A more comple model of vitamin distribution, using pyridoxine as an example (**B**). Regeneration of visual pigment in the eye. Note that 11-*cis*-retinal, a part of the rhodopsin molecule, is enzymatically oxidized and then passes to the retinal pigment epithelium, where it undergoes a series of metabolic steps, returning as 11-*cis*-retinal (*see* text) (**C**). A





map of the distribution of retinol in the body. In the liver, retinyl esters are processed, bind to binding proteins, and are thus exported to blood. Retinol is then passed from one set of proteins to another, from TTR (transthyretin) and RBP in the blood to CRALBP and CRBP in the pigment epithelium (RPE), and then in step 6, to the retina itself with IRBP. The binding proteins stay in their own compartment (D) .

these enzymatic steps. Note, however, that 11-*cis*-retinal formed in the pigment epithelium is first bound to CRALBP (Bok, 1985) and then transferred to IRBP for its return to the retina. The energy needed for the isomerase reaction is derived from membrane phospholipids, an unusual energy source for biochemical reactions (Rando, 1991). In summary, the regeneration of vitamin A for visual pigment function is very complex and involves two different binding proteins and four enzymes within different cells. Intercellular vitamin transport involves retinol, its binding proteins, and probably also specific cell surface receptors for retinol-binding protein (Jones and Helch, 1980).

Since application of 11-*cis*-retinol to bleached rods has a toxic effect on their function, Jones et al. (1989) suggested that IRBP buffers the vitamin so that rods are not exposed to unduly high concentrations. Studies in cultured F9 teratocarcinoma cells with variable expression of the cellular RA-binding protein CRABP-I show that cell lines with high levels of the binding protein are much less sensitive to RA, with less than expected effects on differentiation markers such as laminin B1 and the RA receptor (Boylan and Gudas, 1991). Binding proteins sequester retinoids and reduce their access to receptors and intracellular targets. In vitro experiments show that IRBP helps protect retinol from degradation (Ho et al., 1989). The interplay between retinoids and their binding proteins is complex. Retinal pigment epithelium is readily grown in tissue culture but loses its high content of retinoids after a few days. This occurs only if acceptors, such as albumen, are included in the culture medium (Das and Gouras, 1988).

Hydrolysis of retinyl esters in the cultured RPE is controlled by the presence of acceptors such as IRBP in the medium. If there is no acceptor in the medium, retinyl esters are not hydrolyzed, and retinol is not released. Interphotoreceptor binding protein facilitates the release of retinol and/or retinal from both RPE and outer segment plasma, allowing the retinoid to diffuse to regions of lower concentration. Interphotoreceptor retinol binding protein may also facilitate retinal entry into the outer segments. Figure 1D is a schematic diagram of vitamin A transport from liver to photoreceptor, showing the three different families of binding proteins that are involved. The known major retinoid binding proteins (RBP) are listed in Table 3, including purpurin, the most recently discovered binding protein, which is secreted like RBP (Berman et al., 1987). Cellular retinol-binding protein (CRBP) and cellular RA-binding protein (CRABP) belong to a large family of lipid-binding proteins that also includes the liver fatty acid-binding protein (Napoli et al., 1991).

Vitamin Metabolism and Binding Proteins

All vitamins bind to at least some proteins, and most vitamins with coenzyme functions (Table 2A) are largely protein-bound in tissue homogenates. Folate concentrations within cells are similar to that of folate-utilizing enzymes, i.e., there is almost no free intracellular folate (Schirch and Strong, 1989). Extracellular folates are bound to various folate-binding proteins, and cellular entry is followed by binding to other proteins that use folates as coenzymes (Kane and Waxman, 1989). Ascorbic acid, by contrast, is usually not protein-bound in brain homogenates (Oelrichs et al., 1987) and has higher CSF concentrations than any other vitamin (approx 200 μM in rat and humans). Does the lack of ascorbate protein binding make it more dangerous, or can it be taken as indicating that there is no need for this safety factor? Binding of labeled folates and thiamine to brain membranes in vitro is readily demonstrable and appears not to involve covalent bonds because spontaneous dissociation of the vitamin is easily demonstrated (Snodgrass, unpublished). Tissue homogenates and many cell lines convert retinol into RA. Rates of endogenous RA synthesis may be determined by the ratio of apo to holo CRBP, because the retinol of holo CRBP is recognized by an enzyme with little affinity for free retinol (Napoli et al., 1991).

Table 3
Retinoid Binding Proteins

Protein	Preferred ligand	Mol wt × 1000
Plasma retinol binding protein (RBP)	retinol	21.0
Cellular retinoid binding protein (CRBP)	retinol	16.6
Cellular retinoic acid binding protein (CRABP)	retinoic acid	16.3
Cellular retinaldehyde-binding protein (CRALBP)	retinal	33.0
Interphotoreceptor binding protein (IRBP)	retinol,11-*cis*-retinol, others	126.0
Purpurin	retinol	20.0

This table is modified from Bok (1985). Multiple isoforms of several of the proteins have been described.

Vitamin Entry into the CNS

Many enzymatic and transport reactions are involved in the processing of vitamins within the body. Mutations involving any of these reactions might produce vitamin-dependency syndromes, in which patients require increased vitamin intake to remain healthy. Table 2 also lists those vitamins believed to serve as antioxidants. Although the concentration of most vitamins in CSF (including ascorbate) exceeds that of serum, serum antioxidant activity greatly exceeds that of CSF (Stocks et al., 1974). Since CSF ascorbate concentration exceeds that of serum and vitamin E concentrations are much less (Vatassery et al., 1991), one may infer that ascorbate is a minor contributor to serum antioxidant activity. Vitamins may have more important antioxidant roles in certain intracellular compartments than in the extracellular fluids.

Noncoenzyme Functions of Vitamins

Several vitamins have been found to have additional effects beyond their traditional coenzyme or antioxidant roles. Such additional functions include regulation of gene expression, which is well established for retinoids (a chemical family that includes vitamin A; Sporn and Roberts, 1985; *see* Fig. 2), and similar, albeit less well established effects of vitamin D_3, protein glycation effects, and membrane effects produced by folates and thiamine, which are poorly understood. If PALP is a biological regulator of glucocorticoid receptors, as suggested (Maksymowych et al., 1989), it must also be classified as having both gene expression and traditional coenzyme effects.

Protein glycosylation is an important post-translational modification that is most evident in diabetics and correlates with the extent of diabetic complications in individual patients. This glycosylation involves binding of the aldehyde form of glucose or other sugars to amino groups of proteins to form Schiff bases (Harding, 1985). Less well known is the fact that many nonsugar aldehydes and ketones react with proteins in vivo. For example, various pyridoxal compounds react with hemoglobin in vitro, even in intact cells, changing the oxygen binding curve (Benesch and Benesch, 1981). Table 4 presents a list of known vitamin-induced post-translational protein modifications. Glycation is the covalent addition of a sugar or sugar derivative to a protein, generally without enzymatic catalysis. Note that RA, a retinol metabolite, biotin, and ascorbate are capable of such reactions, which probably alter the function of the protein that is modified. This protein-modifying potential of carbonyl-containing compounds is rarely significant when the compound is present at low concentrations and is protein-bound, but it may become important at supranormal vitamin concentrations, especially if the capacity of binding proteins is exceeded. This modification of protein structure may be harmful, beneficial, or without functional effect, depending on the protein involved and the specific circumstances. Khatami et al. (1988) reported that PALP, ascorbate, and dehydroascorbate all inhibited nonenzymatic glycosylation of bovine

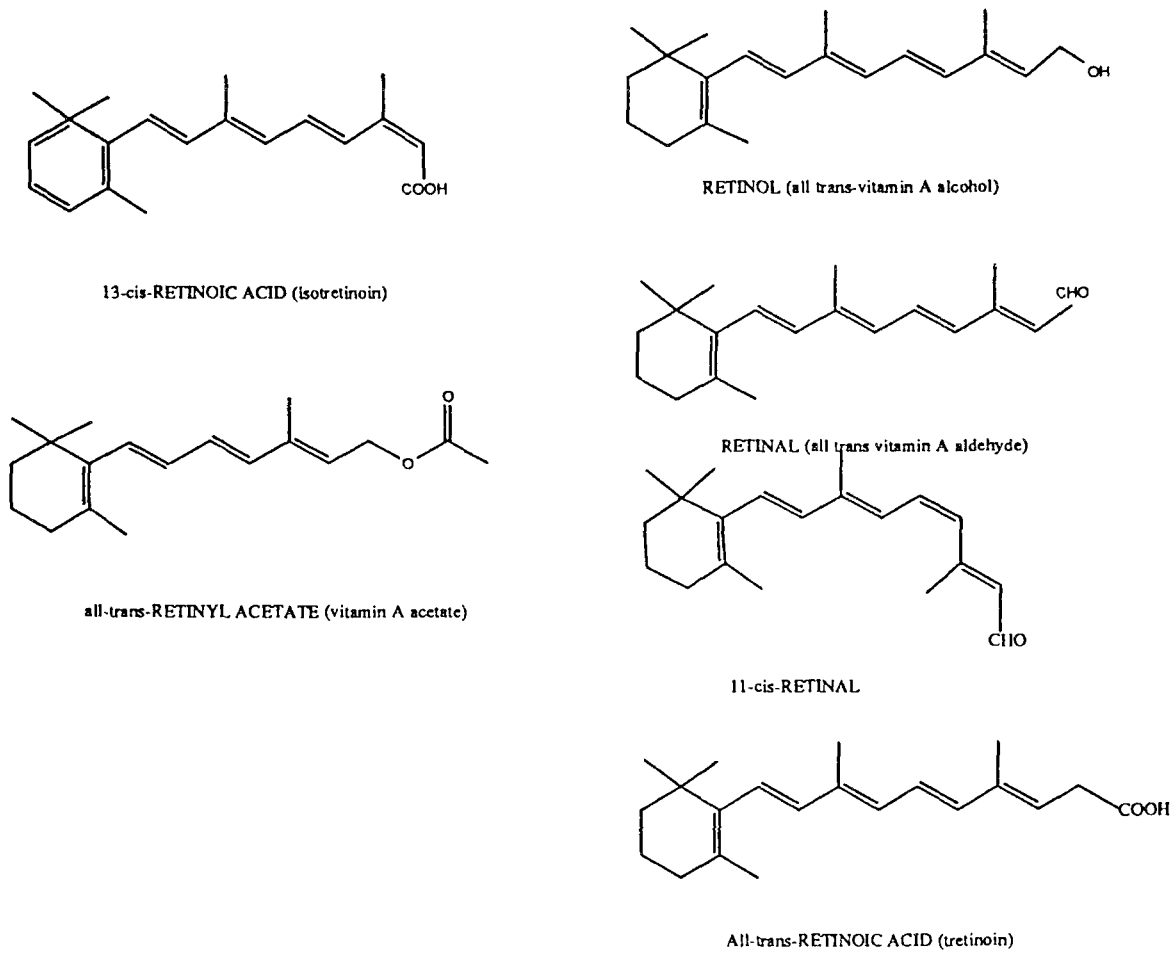


Fig. 2. Structure of some of the most important retinoids.

serum albumin (BSA) in vitro, implying possible benefit for diabetic patients. The possibility of injury from such vitamin-induced glycation has been largely ignored (Dunn et al., 1990).

Identification of Vitamin Neurotoxicity

The Importance of Animal Studies

The first example of vitamin toxicity was hypervitaminosis A (Bendich and Langseth, 1989). Whereas most cases of vitamin A toxicity are attributable to excessive vitamin intake, well documented cases have been caused by ingestion of large quantities of carnivore liver, livers of large fish, and even chicken liver. This is because large amounts of vitamin A are stored in the livers of many animals. The clinical literature is replete with reports of patients whose symptoms have been ascribed to vitamins, medication, or other factors, without documentation of a causal relationship (e.g., showing that symptoms remit when the vitamin is stopped and return after vitamin resumption). In addition to the possibility that a vitamin was unrelated to the symptoms, there is the additional problem of quantitation of vitamin intake. Patient reports of vitamin intake are often inaccurate, and many patients take

Table 4
Known Protein Modification Reactions Involving Vitamins

Reaction	Enzymatic basis?	Group involved	Reference
Retinoylation	yes	?thioester bond to protein	Renstrom and DeLuca, 1989
Pyridoxylation	no	Schiff base reaction with amino groups	Harding, 1985
Biotinylation	no	similar to above	—
Ascorbate induced	no	amino group of lysine	Dunn et al., 1990

multiple food supplements, making it difficult to know which, if any, might be causally related to an illness.

Although supranormal plasma levels of a vitamin suggest excessive vitamin intake and lend credence to the possibility that symptoms may be vitamin-related, we may not assume that normal plasma levels of a vitamin rule out neurotoxicity. Although rare, such occurrences have been reported (Mendoza et al., 1988) and suspected in siblings who differed greatly in vitamin A tolerance (Carpenter et al., 1987). Smith and Goodman (1976) pointed out that the ratio of plasma vitamin A to RBP was the single best indicator of vitamin toxicity. Mutations of vitamin "receptors," vitamin binding proteins, or vitamin metabolizing enzymes might lead to vitamin neurotoxicity without unusual vitamin intake or plasma levels. Under conditions of malnutrition and hepatic injury, synthesis and release of plasma-binding proteins (such as rRBP) may be impaired so that the amount of free vitamin is excessive in spite of "normal plasma levels" (Mendoza et al., 1988). Since bound vitamin is usually less reactive than free vitamin, measures of free vitamin content or of vitamin binding capacity increase the information provided by a "plasma vitamin level."

Controlled experimental studies are the best way to identify toxic vitamin effects in animals but are subject to important species differences, which are most obvious in the case of ascorbate. Controlled studies of the effect of vitamin consumption on subtle human brain functions are difficult because of problems in monitoring dietary intake for periods of weeks or months and the need for repeated objective assessments of performance. Such studies are expensive and have difficulty competing for research funding. Until such studies are done, we must combine evidence from animal studies with the limited and imprecise information available about human vitamin toxicity. This review focuses on documented human vitamin neurotoxicity and potential neurotoxicity suggested by animal studies.

Toxic Effects of Vitamin Contaminants

The use of vitamin supplements by healthy and sick adults has increased greatly over the past 25 years in the US, and some studies indicate that as many as 50% of adults regularly take vitamin supplements, some in high dosage. Manufacture, quality control, and storage of vitamins is not free from difficulties. The recent epidemic of eosinophilia-myalgia syndrome in persons taking large quantities of tryptophan appears to be caused by a contaminant in the production of tryptophan (Mayeno et al., 1990) and points up the need for attention to even minor contaminants if a substance is taken repeatedly in supraphysiological amounts. No such contaminant has been identified as responsible for vitamin neurotoxicity, but little has been done to investigate this possibility. In the US, the Food and Drug Administration (FDA) is responsible for overseeing the safety of food supplements, such as vitamins, but does not perform routine analyses of vitamins and requires only that vitamin preparations be 98% pure.

Permeability Factors

Transport systems for water-soluble vitamins have been extensively studied (Rose, 1988). Spi-

nal fluid levels of many exceed plasma levels, implying some kind of selective permeability or transport (Spector, 1977). Although intraventricular injection of any vitamin in large doses will probably disrupt brain function, systemic megavitamin administration usually has little effect on brain vitamin content. For example, Cohen et al. (1973) found that a 200-fold increase in dietary pyridoxine intake had no significant effect on rat brain vitamin levels. Experimental rats had higher hepatic pyridoxine and pyridoxal concentrations than controls, but the content of active coenzyme (pyridoxal and pyridoxamine phosphate) was no different than controls. We know that the CNS is much less influenced by fluctuations in vitamin intake (and contaminants present in commercial vitamin preparations) than are other organs.

Table 5 presents data from an experiment comparing the effects of iv and systemic pyridoxine administration on brain content of PALP and shows that the ratio of pyridoxal to PALP increases markedly after intraventricular but not after systemic administration. We postulate that high tissue ratios of pyridoxal to PALP indicate increased potential for toxicity owing to nonspecific pyridoxylation of proteins by pyridoxal (assuming that PALP is less likely to cause nonspecific pyridoxylation). If the ratio of vitamin to its binding or carrier proteins is critical in determining tissue toxicity, as argued by Smith and Goodman (1976), injection of vitamins directly into brain or CSF is hazardous because the liver, site of synthesis of most plasma binding proteins, receives no message for increased synthesis and release of binding proteins.

Table 5
Pyridoxal and Pyridoxal Phosphate Concentrations After Systemic or IV Administration

Systemic injection of pyridoxine (100 mg/kg)			
	PN	PAL	PALP
Liver	1.65 + 0.19	6.22 + 0.85	3.15 + 0.46
Brain	0.02	0.31 + 0.04	2.15 + 0.38
IV injection	(50 µg)		
Brain	0.21 + 0.04	3.35 + 0.70	1.86 + 0.47

In each case, rats were sacrificed 18 h after injection. The brains were removed and homogenized in 0.1*M* perchloric acid, and the content of pyridoxine (PN), pyridoxal (PAL), and pyridoxal phosphate (PALP determined by the chromatographic-fluorometric method of Loo and Badger (1969). The units are nmol/pyridoxine or pyridoxal per g wet wt of tissue. Four rats were included in each study. Brain vitamin levels were not significantly different from controls in the systemically injected rats, but there were significant increases in content of each vitamer, and the ratio of PAL/PALP changed from 0.24 (controls) to 1.97 (systemic injection).

Vitamin Interactions with Drugs and Other Vitamins

Additional complexities arise when a vitamin is coadministered with an active drug. Pyridoxine forms Schiff bases with nitrogenous compounds, usually reducing their pharmacological effects. It also changes the metabolism of drugs that are decarboxylated in the body. Coadministration of L-DOPA and pyridoxine has long been known to decrease DOPA effects (Duvoisin et al., 1969). This antagonism is well explained by peripheral pharmacokinetic factors without the need of postulating CNS actions of the vitamin. We have already mentioned that vitamin E or other reducing agents may sometimes substitute for ascorbate and reduce the impact of dietary ascorbate deficiency. Other, more complex interactions between vitamins exist, such as the ability of ascorbate to increase tissue cobalamin content in B12-deficient rats (Thenen, 1989). Another example of subtle interaction between vitamins is that the synthesis of CRBP type II (Table 3) is controlled by vitamin D_3 (Finlay et al., 1990).

Ascorbate markedly prolongs barbiturate sleeping time in mice and decreases pentobarbital metabolism (Hollinshead et al., 1990). Methylation of mercury compounds increases their ability to enter the CNS and also prolongs their stay in the body. Since mercuric ions can be biomethylated by cobalamins, persons taking large doses of folate and B12 may be more likely

to be harmed by mercury should they contact it. Guinea pigs injected with megadoses of ascorbate, B12, or folate showed increased mercury methylation in brain and muscle, although clinical neurotoxicity was not seen (Zorn and Smith, 1990).

Neurotoxicity of Specific Vitamins

Vitamin A

Toxicity of Vitamin A and Analogs

Vitamin A was chemically characterized in 1931. Many compounds are known to have some vitamin A-like activity. β-ionone derivatives with activity similar to that of all-*trans* retinol are considered to have vitamin A activity. They include retinol, retinal, and various retinyl esters (Fig. 2). Vitamin A is ingested in the diet as retinyl esters, which are transported to the liver with chylomicrons. They are hydrolyzed in hepatic parenchymal cells, secreted with RBP and selectively taken up by hepatic stellate cells. In the plasma, retinol and RBP circulate as a complex with transthyretin or prealbumin (*see* Fig. 1D). Retinol binding protein and transthyretin do not enter the brain or retina. Excess vitamin A is converted to retinyl esters again and stored in the liver, hence the potential of animal liver to produce hypervitaminosis A. The structure of various retinoids is shown in Fig. 2.

Retinol was first shown to be essential for retinal function, hence its name (Sporn and Roberts, 1985). However, it has additional effects on skin and other tissues. Retinoic acid is formed in the body from retinol and shares some of its biological actions (Wolf, 1990). In certain systems, retinol and RA are similar in biological potency (Bagavandoss and Midgley, 1987). Recent years have brought important developments related to retinoids: The identification of multiple RA receptors in many tissues, with strong evidence that RA is a major controller of differentiation (Petkovich et al., 1987), based in part on the discovery of nuclear RA receptors that influence transcription of other genes (Blomhof et al., 1990); and the use of vitamin A analogs, primarily 13-*cis* RA or isotretinoin, in the treatment of acne and other skin diseases (Leyden, 1988).

Retinoic acid has profound effects on the differentiation state of cultured cells (Collins, 1987) and is a potent teratogen in whole organisms (Durston, et al., 1989). Changes in neurofilament proteins are prominent during RA-induced differentiation (Hall et al., 1990), and RA also alters protein kinase C (PKC) activity (Slack and Proulx, 1990), and membrane Ca^{2+} ATPase activity (Davis et al., 1991). Retinoic acid induces the synthesis of the transcription factor AP-2 (Luscher et al., 1989) and prevents the development of experimental autoimmune encephalomyelitis in rats (Massacesi et al., 1991). We have found RA effects on ^{45}Ca fluxes and calcineurin enzyme activity in CNS tissues (Table 6). Calcineurin is a calcium-dependent protein phosphatase that often acts to reverse phosphorylation catalyzed by cAMP-dependent kinase (Sharma and Wang, 1985). A recent report (Alcalay et al., 1991) indicates that many patients with acute promyelocytic leukemia have chromosome translocations involving the RA α-receptor gene (RAR), predicted to disrupt the *N* terminus of the protein. Alcalay et al. also suggest that the abnormal RAR α-protein responds abnormally to endogenous retinoids with consequent disruption of normal differentiation programs. Patients with acute promyelocytic leukemia have been successfully treated with tretinoin (all-*trans*-RA) (Warrell et al., 1991). All patients who responded had expression of aberrant RAR.

Isotretinoin is very effective for the treatment of acne but also has major CNS teratogenic effects (Lammer et al., 1985). A constellation of characteristic defects in infants exposed to isotretinoin in early pregnancy includes craniofacial, CNS, cardiovascular, and thymic malformations. Many of the anomalies are severe and cause death or massive handicap. These teratogenic effects are readily understood if one compares the structure of isotretinoin and RA and considers that RA is a

Table 6A
Vitamin Effects on ^{45}Ca Fluxes and Calcineurin Activity in Neural Tissues

^{45}Ca Fluxes in C6 cells		
Vitamin	Ratio of experimental to control	^{45}Ca influx
Biotin	20 μM	1.07 + 0.14
Biotin	400 μM	2.16 +0.25*
Retinoic acid	25 μM	1.79 + 0.21*
Retinol	200 μM	1.53 + 0.16*
Folic Acid	400 μM	1.95 + 0.18*
Folic Acid	1000 μM	2.29 + 0.40*
Methotrexate	400 μM	2.85 + 0.33*
Pyridoxal	25 μM	1.08 + 0.24
Pyridoxal	250 μM	1.75 + 0.28*
Pyridoxine	250 μM	1.29 + 0.30
Thiamine	250 μM	1.15 + 0.31
Thiamine	1000 μM	0.90 + 0.19

*Cultured C6 glioma cells were freed from growth medium, suspended in a HEPES-buffered salt solution containing 10 m*M* glucose, and preincubated for 30 min, after which concentrated vitamin solutions were added to give the final concentrations shown and the incubations continued for 20 min at 30°C. Retinol and retinoic acid were added from DMSO solutions, and controls therefore contained 2.5% DMSO. Control values (+SEM) for ^{45}Ca uptake were 1.04 + 0.09 nmol/mg prot/incubation and 1.15 + 0.13 nmol/mg prot for DMSO controls. *-the difference between this value and that of the control group was significant at $p < 0.05$ by Shaffer's modification of Dunnett's test (Shaffer, 1977).

Table 6B
Calcineurin Enzyme Activity in Brain Slices

Vitamin	Mean enzyme activity
Control	6.3 + 0.8
Control + 1 m*M* ascorbate	6.0 + 0.7
Retinoic acid, 400 μ*M*	10.8 + 2.3*
Cholecalciferol, 400 μ*M*	13.5 + 3.3*
Folic acid, 1 m*M*	9.7 + 1.5*
Methotrexate, 1 m*M*	12.0 + 2.4*
Pyridoxal, 250 μ*M*	7.3 + 1.0

*Slices (0.25 mm) from the forebrain of adult rats were incubated for 20 min at 35°C with the vitamin concentrations shown, after which the slices were homogenized in Tris HCl 25 m*M*, pH 7.4, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, and calcineurin enzyme activity determined by the spectrophotometric method of Pallen and Wang (1983). Each group contains five flasks of slices, each flask containing about 20 mg wet wt of brain tissue. Folic acid and retinoic acid contained ascorbate in the incubation buffer. Values given are + SEM and the units are nmol phosphate released/mg protein/10 min incubation period with the artificial substrate *p*-nitrophenylphosphate.

major regulator of gene expression. In addition to its usefulness for treatment of acne, isotretinoin has also been shown to reduce the frequency of development of new primary tumors in a group of patients with squamous-cell carcinoma of the head and neck (Hong et al., 1990). Isotretinoin has also produced pseudotumor cerebri and oculogyric crises, similar to the symptoms of hypervitaminosis A (Bigby and Stern, 1988). Doses of vitamin A only slightly in excess of the RDA produce neurological toxicity in some cases (Farris and Eerdman, 1982). Vitamin A and related fatty acids have long been known to produce membrane injury at high dosage (Meeks et al., 1981). Long-chain fatty acids and certain retinoids with a 13-*trans* configuration inhibit red cell membrane Ca^{2+} Mg^{2+} ATPase (Davis et al., 1991). Folates and some retinoids have similar effects on brain membranes, but the concentrations required are greater (Snodgrass, unpublished).

Given the increasing number of biological effects mediated by retinoids, it is surprising that vitamin A and retinoids do not cause trouble more often. Their ready penetration into the CNS (Kalin et al., 1982), unlike many other vitamins (i.e., pyridoxine, folates, and ascorbate), suggests a greater potential for CNS side effects. Small children probably tolerate proportionately less vitamin A than adults (Hathcock et al., 1990). In some cases, children have developed serious vitamin A neurotoxicity from diets rich in chicken liver (Mahoney et al., 1980). Figure 3 presents a diagram, taken from Wolf's paper, showing the nuclear actions of vitamin A and RA and the special role of RA receptors in these actions. Recall that all the actions shown in Fig. 3 are non-coenzyme actions, separate from the coenzyme role of retinol in the eye, described in Fig 1C.

The steroid receptor superfamily can be divided into two general subclasses. The first and most numerous includes glucocorticoid, miner-

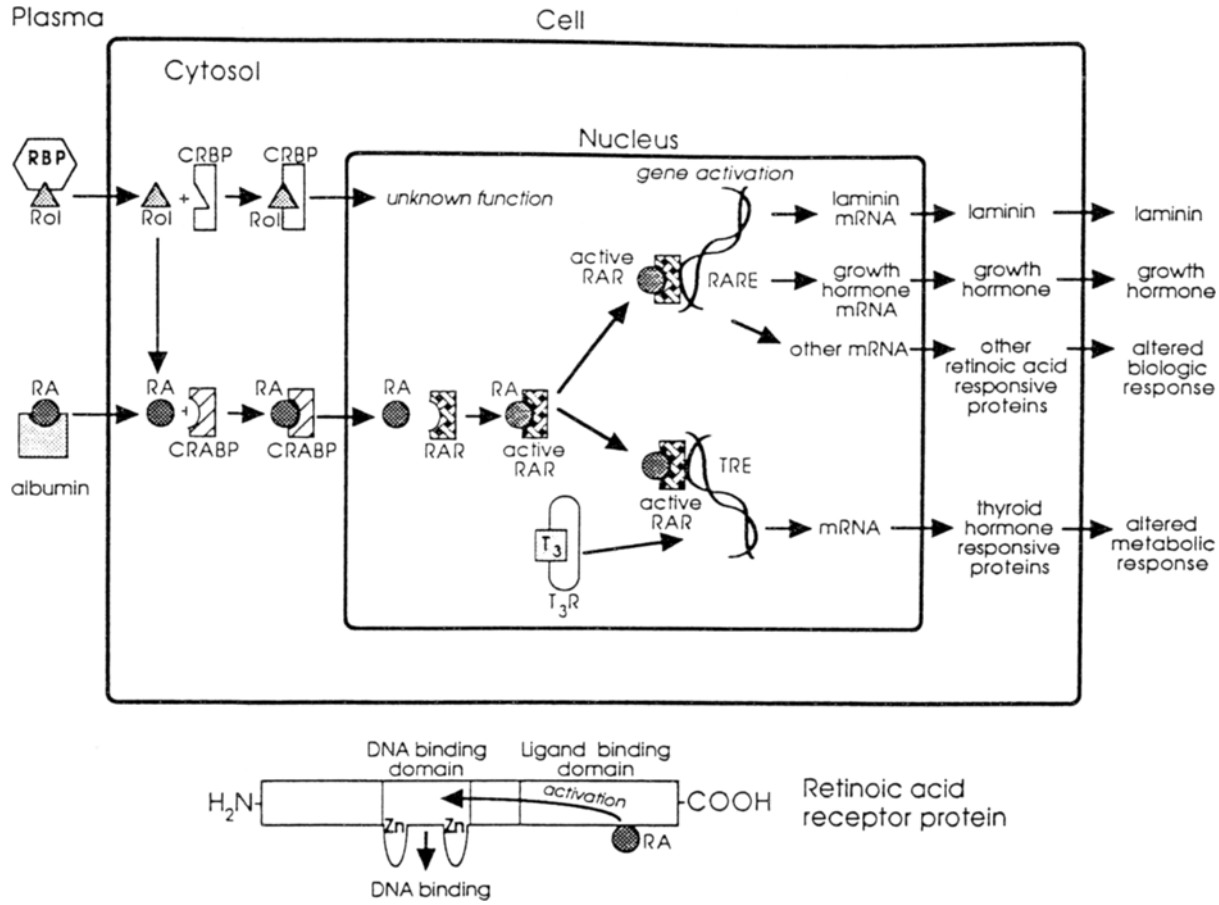


Fig. 3. Simplified diagram of the genomic effects of retinoic acid. RBP, serum retinol binding protein; Rol, retinol; RA, retinoic acid; CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid binding protein; RAR, retinoic acid receptor; RARE, retinoic acid-responsive element on a hypothetical gene (part of the promoter region that binds RR-RAR with resulting increase in rate of transcription); TRE, thyroid hormone-responsive element; T_3, thyroxine; T_3R, thyroxine receptor; mRNA, messenger RNA. This figure reproduced from Wolf (1990), with permission.

alocorticoid, estrogen, androgen, and dioxin receptors (Dalman et al., 1991). All these receptors are found in the cytosol after hypotonic cell lysis, bind to heat shock protein (hsp90; Perdew, 1988), and remain in an inactive "docking complex" until bound ligand evokes changes that produce high-affinity association with nuclear components followed by transcriptional activation. The second group of receptors include RA and thyroid hormone receptors. For this group, unliganded receptors are recovered in the nuclear fraction, receptors do not bind hsp90 and do not form "docking complexes" (Dalman et al., 1991).

Hypovitaminosis A, isotretinoin, and excessive vitamin A all cause the syndrome of pseudo-tumor cerebri (Ahlskog and O'Neill, 1982). This condition responds well to vitamin wishdrawal (restoration) once it has been recognized and its cause identified. However, patients may suffer permanent loss of visual acuity if the condition persists for long periods of time. It is usually accompanied by headache, but the headache may not be dramatic. Furthermore, many of the cases described have been in infants unable to complain specifically of headache (Marie and See, 1954). The effects of hypervitaminosis A in rat pups

differ in different organs and at different ages (Shukla et al., 1983), but there is no doubt that brain cell number is reduced by vitamin A excess in the rat pup.

Hypovitaminosis A

Vitamin A deficiency is very common in underdeveloped countries. Vitamin A is surprisingly potent in reducing death caused by infection in malnourished children in Asia and Africa (Keusch, 1990). The fact that hyper- and hypovitaminosis can have similar or identical symptoms is surprising. However, premature differentiation and failure of differentiation might both lead to similar outcomes, i.e., a shortage of cells with a particular function. The analogy with amyloid β-protein may be instructive. This protein has been reported to be both neurotoxic and neurotrophic in cultured hippocampal neurons. A recent study found that low concentrations of the protein (ED_{50} 0.6 n*M*) had trophic effects, whereas high concentrations (ED_{50} 100 n*M*) evoked toxic effects. Both effects were blocked by tachykinins and seemed to depend on the same domain of the protein (Yankner et al., 1990).

Ascorbate

Scurvy was the first vitamin deficiency state to be recognized, in the 18th century. Ascorbic acid was shown to protect humans against scurvy in the early 1930s, but its function remains poorly understood today (Englard and Seifter,1986). Reducing equivalents donated by ascorbate are important in several reactions, including the hydroxylation of dopamine to norepinephrine by the enzyme dopamine-β-hydroxylase, responsible for norepinephrine synthesis. Amidation of neuropeptides by the recently discovered peptidylglycine α-amidating monooxygenase may also explain the high ascorbate concentrations found in adrenal, brain, and pituitary (Padh, 1990). No strong binding between ascorbate and any of the enzymes thought to be as ascorbate-dependent has been found. The vitamin serves to keep metal ions in these enzymes in a reduced state and other reducing agents can often substitute. Suggestions of Pauling and others that megadose supplements of ascorbate might protect against certain common diseases, ranging from the common cold to cancer and mental illness, have generated much discussion but little supporting evidence.

Ascorbic acid is an important antioxidant in humans that is maintained in the reduced state in tissues as well as plasma. Interaction of ascorbate with free radicals in the body results in single electron transfer to R from ascorbate, producing ascorbate free radical. Ascorbate free radical is not thought to be highly reactive with tissue components, nor very dangerous (Rose, 1989). Ascorbate free radical undergoes nonenzymatic dismutation to produce a molecule of ascorbate and a molecule of the fully oxidized dehydro-L-ascorbate, or DHAA (Fig. 4). The more reactive DHAA is structurally similar to alloxan and produces experimental diabetes when given to rodents in high dosage (Patterson, 1950). It may also further oxidize to L-threose, which forms complex glycation adducts with the ε-amino groups of lysine residues in proteins, as discussed below.

Ascorbate concentrations in brain exceed those of most other tissues (Chinoy, 1972). In certain brain regions, the extracellular concentration of ascorbate may exceed 400 u*M* (Stamford et al., 1984). Ascorbate concentrations in brain extracellular fluid vary depending on activity and time of day and correlate rather well with motor activity (O'Neill and Fillenz, 1985). Treatments such as amphetamine or benzodiazepine injections and behavioral treatments such as tail pinch all change striatal extracellular ascorbate concentrations, as monitored by *in situ* voltammetry (Boutelle et al., 1989). Single large doses of ascorbate increase rectal temperature in guinea pigs and humans, probably by stimulating prostaglandin synthesis (Johnston, 1989). Iontophoretic application of ascorbate increased the firing rate of many striatal neurons in anesthetized rats (Gardiner et al., 1985).

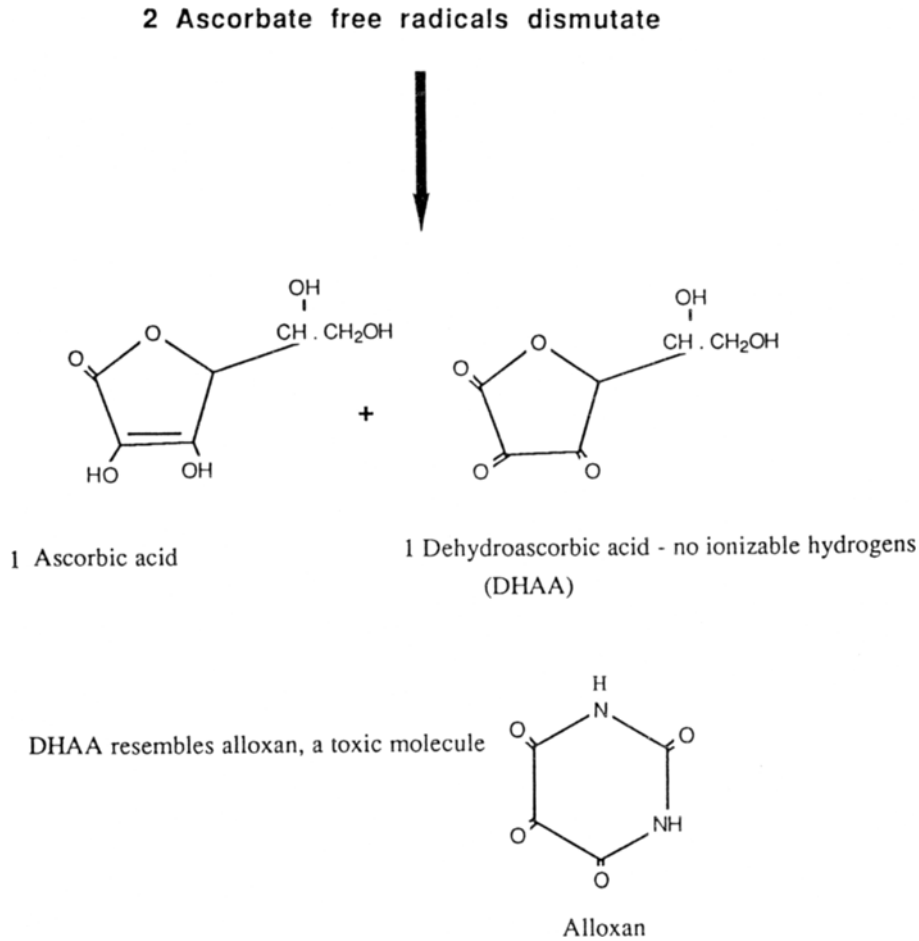


Fig. 4. Dismutation of ascorbate free radical to form ascorbate and DHAA.

Because such studies have reported similar responses to ascorbate and glutamate, it has been suggested that ascorbate may alter glutamate release or cellular responses to glutamate. Specific release of ascorbate by $GABA_A$ agonists from striatal homogenates and minces has been demonstrated, suggesting that this ascorbate release may serve a physiological function (Bigelow et al., 1984). Radioligand binding studies in vitro indicate that the binding of many ligands is ascorbate-sensitive (Muakkasseh-Kelley et al., 1982). Although this was first ascribed to protection of monoamines such as dopamine and serotonin from oxidation (Andresen and Shih, 1986), the effect extends to transmitters insensitive to spontaneous oxidation, and effects on receptor macromolecules (Andorn et al., 1988; Hadjiconstantinou and Neff, 1983) are at least as common as effects on ligands. Ascorbate may have different effects on membrane lipid peroxidation depending on its concentration (Muakkasseh-Kelley et al., 1982).

Systemic ascorbate administration slows complex reaction time in humans (Benton, 1981), synchronizes the electroencephalogram (EEG), increases the motor activity of rats (Wambebe and Sokomba, 1986), potentiates the stimulatory effects of amphetamine, and opposes the effects of pentobarbital and haloperidol in rats. Although it potentiates amphetamine effects,

ascorbate alone may have mild sedative effects in humans (Benton, 1981) and has neuroleptic-like effects when given in high doses to rats (Pierce et al., 1991). In vitro studies show that ascorbate alters 2-deoxyglucose accumulation in cultured neural cells and changes the response to phorbol ester treatment, suggesting that it can exert important metabolic effects (Table 7). Note the biphasic relationship between ascorbate concentration and effect, suggesting that large doses of ascorbate might have effects opposite to those of small doses.

These considerations suggest that ascorbate is an active and potentially dangerous molecule. However, many humans have taken supraphysiological quantities of ascorbate for years without obvious harm. Increased ascorbate intake may increase the risk of kidney stones (Chalmers et al., 1986). Although supranormal intake of ascorbate may have some beneficial effects on skin flaps and wound healing (Hayden et al., 1987), ascorbate enhances iron-catalyzed lipid peroxidation under certain circumstances (Chen and Chang, 1979; Stadtman, 1988) and may worsen outcome after hypoxia (Hershko et al., 1987).

The in vivo effects of large doses of ascorbate are difficult to predict from in vitro studies. Sodium ascorbate increased the ability of sodium saccharin to produce bladder cancers in rats, whereas ascorbic acid did not (Fukushima et al., 1990). Several studies of this phenomenon suggest that the cancer-promoting effect of ascorbate is evident only at high urinary pH, which is abolished when ascorbic acid is used. Protein glycation by ascorbate and other vitamins might retard deleterious glycosylation by glucose, often suspected of contributing to cellular aging. This would be a beneficial noncoenzyme vitamin effect. However, incubation of ascorbate and proteins without glucose results in the formation of glycation products (Dunn et al., 1990), indicating that ascorbate-induced protein modification might be harmful independent of glucosylation.

These studies of carcinogenesis, lipid peroxidation, and so on refer to non-CNS effects of ascorbate. Table 7 shows that the effects of higher concentrations of ascorbate are modified if glutathione is also included, to maintain ascorbate in a reduced state (Winkler, 1987). Large doses of ascorbate may be safe if combined with additional reducing agents and dangerous if they are not. Like pyridoxine, ascorbate may alter the effects of CNS-active drugs and was shown to prolong barbiturate sleeping time in mice (Hollinshead et al., 1990).

Injection of ascorbate directly into the cisternal spinal fluid has been advocated in the treatment of patients with subarachnoid hemorrhage owing to ruptured aneurysms (Kodama et al., 1986). Hemorrhage from cerebral aneurysms is often followed by the development of cerebral vasospasm, with resultant decreased brain perfusion, worsening of patient status, and even death. Such vasospasm may be explained by the actions of oxyhemoglobin, produced by the hemorrhage, on cerebral blood vessels (Boullin et al., 1983). Reduction of oxyhemoglobin with ascorbate in vitro reduces its vascular effects (Kawakami et al., 1991). This has led to the supposition that perfusion of patients with ascorbate and urokinase would improve outcome. No control group was used in the Japanese study, but no ascorbate side effects were recognized. One may wonder about how closely the patients were monitored, since ascorbate infusion into the cisterna magna should alter the surface proteins of brain stem cells nearby (Levine, 1983). There is no way to predict *a priori* whether such changes would be beneficial or harmful. If untoward effects occurred, there might be a tendency to ascribe them to effects of the preceding hemorrhage. Only controlled studies can clarify this issue.

Furthermore, little ascorbate would be expected to reach the site of aneurysmal bleeding, which is usually beneath the frontal lobes near the circle of Willis (Asbury et al., 1986). Drugs injected into the cisterna magna tend to be distributed over the cerebral hemispheres rather than going into the ventricles or along the undersurface of the brain (Poplack et al., 1980). Such therapy is potentially hazardous and should

Table 7
Ascorbate Effects on Deoxyglucose Accumulation by Cultured C6 Cells

Treatment	Incubation time	SEM (pmol/mg prot)
Control salt solution	30 min	319 + 44
Ascorbate 200 μM	30 min	304 + 36
Ascorbate 2 mM	30 min	353 + 40*
Ascorbate 5 mM	30 min	389 + 51*
Ascorbate 10 mM	30 min	265 + 27*
Ascorbate 10 mM + glutathione 2 mM	30 min	415 + 57*

*Each group included 5–7 wells of cultured C6 cells, grown until confluent, after which growth medium was aspirated and replaced by HEPES-Ringer solution containing the indicated concentration of sodium ascorbate. After a 10-min preincubation, ^{3}H-2-deoxyglucose was added to a final concentration of 90 nM and the cells incubated for 40 min at 37°C. Cells were washed with buffer, lysed with 0.2M NaOH-0.2% Triton, and retained radioactivity was determined by scintillation counting.

not be employed until studies with control groups show that patients receiving CSF vitamin infusions do better than controls and establish a safe dose of ascorbate.

Many membrane proteins are sensitive to tissue redox state (Levine, 1983). The *N*-methyl-D-aspartate receptor, thought to be involved in neuronal damage owing to seizures, ischemia, and other pathological states (Choi, 1988), is sensitive to redox manipulations. It is inhibited by ascorbate, whereas reductants such as dithiothreitol (DTT) and penicillamine, which break protein disulfide bonds, potentiate receptor function (Majewska et al., 1990). Since there is evidence of ascorbate release into the cerebral extracellular fluid from ischemic brain (Hillered et al., 1988), one could speculate that released ascorbate might improve survival in cerebral ischemia, preventing extension of the injury by excitatory amino acids acting at NMDA receptors (Rothman and Olney, 1987). Ascorbate effects on oxidation-reduction state of proteins are complex: In some systems, ascorbate and DTT have similar effects (Levine, 1983) and may operate by reducing Fe^{3+} ions. In other systems, such as the NMDA receptor (Majewska et al.,1990), their effects are opposite, and Fe^{3+} ions are probably not involved. Infusion of exogenous ascorbate after the injury would probably be ineffective because it would not reach the site of ischemia, even if its in vivo effects were desirable, which is unproven.

Folates

Folates are derivatives of folic acid (FA), which itself is rarely found in the mammalian body. Reduced folates are stored as polyglutamates inside cells (Schirch and Strong, 1989), hydrolyzed to monoglutamates in the intestine, and concentrated in the liver, where they are transformed in several ways (Cook and Blair, 1979). The primary folate of vertebrate extracellular fluids is 5-methyltetrahydrofolate. Folate was pressed into use for treatment of megaloblastic anemias soon after its synthesis in 1945. Anecdotal reports of neurological symptoms associated with folate use and deterioration of neurological function in folate-treated patients with pernicious anemia soon appeared (Editorial, 1947). We still do not know whether folates cause deterioration in pernicious anemia, as suggested by the editorial, or simply act to mask the problem by stimulating hematopoiesis without helping the CNS problem. Because folates may be harmful for the neurological status of patients with pernicious anemia, they are excluded from multivitamins in some states.

For more than 20 years, injections of FA in large doses have been known to cause toxic nephro-

pathy (Thomas and Mayfield, 1972). By the 1970s, folates had been shown to produce seizures in several animal species. Seizures require high doses of folates; most experimenters have injected folates directly into the brain or into the CSF. A consistent potency ratio for seizures and related neurotoxic effects has emerged: Folic acid is more toxic than 5-formyltetrahydrofolate, which is more potent than 5-methyltetrahydrofolate (Olney et al., 1981; Van Rijn et al., 1990). Most vertebrate cells, possibly all, make high-affinity, folate-binding proteins (Kane and Waxman, 1989), some of which bind "unnatural" or oxidized folates with higher affinity than 5-methyl and 5-formyltetrahydrofolate, the primary folates of vertebrate extracellular fluids. These folate-binding proteins may have a role in folate transport and are found in brain and spinal cord. Folates are known to serve as coenzymes for nine cytosolic and four mitochondrial enzymes (Schirch and Strong, 1989), but these coenzyme effects cannot explain their excitant effects on neurons.

Hommes and coworkers (1977) reported that changes in dietary folate content produced inverse effects on the seizure thresholds of rats, as determined with pentylenetetrazol. Small effects of dietary folate on the activity of brain cholinergic and GABAergic enzymes have been reported (Botez, 1980). Folate injections directly into the brain or spinal fluid cause seizures (Obbens and Hommes, 1973). Olney and colleagues (1981) studied the seizures and histological damage resulting from intracerebral folate injections, noting similarities to those produced by the neurotoxin kainic acid, which is much more potent. Although numerous anecdotal reports indicate increased frequency of seizures in human epileptic patients given folates, most controlled studies have found no difference (Gibberd et al., 1981). Ch'ien et al. (1975) studied the EEG effects of folate infusions in eight epileptic patients and noted that one had worsening of her EEG without clinical manifestations and another, who received a smaller dose, had convincing EEG changes and clinical seizures after infusions of 14 and 19 mg. The patient who had two folate-evoked seizures received less folate than any of the other patients, and the authors commented on large individual differences in folate sensitivity. Exactly how folates cause seizures remains unknown. There is some evidence that folates alter GABA receptor function in hippocampal slices (Otis et al., 1985) and ligand-binding studies (Van Rijm et al., 1990), and the potency ratio of folates for altering the binding of the chloride channel ligand TBOB (t-butyl bicyclo-orthobenzoate) is the same as the potency ratio for in vivo excitotoxicity.

More than half of the patients with megaloblastic anemia owing to B12 or folate deficiency have some form of neuropsychiatric complication. Demented and psychotic patients seem to have more folate deficiency than control populations, and some have suggested that folate deficiency contributes to mental illness. A recent British study found that addition of 5-methyltetrahydrofolate to standard psychiatric drug treatments improved the outcome of depressed and schizophrenic adult patients (Godfrey et al., 1990). The authors believe that this is a CNS effect.

The slime mold *Dictyostelium discoideum* feeds on bacteria and shows chemotactic responses to folates secreted by the bacteria (Pan et al., 1975). Biochemical studies show that folate produces rapid increases in cGMP in slime molds (De Wit and Bulgakov, 1985) and stimulates ^{45}Ca influx (Milne and Coukell, 1991). The membrane receptor responsible for these folate responses is coupled to an unknown GTP-binding protein and is more sensitive to folic acid than reduced folates. It is also responsive to pteridines (pteridines are part of the folate molecule). The author has speculated that membrane proteins related to the *Dictyostelium* folate receptor may exist in mammals and explain the cGMP and calcium flux effects that are produced by folates in high doses (unpublished; *see also* Table 6).

Pyridoxine and B6 Vitamers

Vitamin B6 is a generic name for all 2-methylpyridine derivatives with biological activity. The major coenzyme metabolite of this group of compounds is pyridoxal-5-phosphate. It serves as a coenzyme for transaminations, decarboxylations, deaminations, and other enzymatic reactions important in amino acid metabolism. As indicated in Table 3, nonspecific binding of pyridoxine to amino groups of peptides, drugs, and so on (Schiff base formation) is known to occur and to alter the functional state of macromolecules that have bound pyridoxine groups. In addition, Litwack and coworkers reported more than 10 years ago that m*M* concentrations of PALP altered hepatic glucocorticoid receptor function, changing both DNA binding by the activated, liganded receptor and binding of steroid ligands (Maksymowych et al., 1989).

The biological relevance of this observation was strengthened by studies in cultured cells showing that manipulations of PALP content altered gene expression (induction of the enzyme tyrosine aminotransferase; DiSorbo and Litwack, 1981) and more recent studies suggesting that μ*M* concentrations of pyridaxal phosphate had significant effects on DNA binding of purified glucocorticoid receptors. Hence, Litwack and coworkers have claimed that "PALP is a biological regulator of the glucocorticoid receptor system" (Maksymowych et al., 1989). This is supported by studies of glucocorticoid receptor responses in HeLa cells (Allgood et al., 1990). Evidence is increasing that PALP influences glucocorticoid receptor responses, often without effects on the amount of receptor protein or mRNA.

Pyridoxine is one of three vitamins known to produce convulsions if given in large doses; the others are folates and thiamine. Pyridoxine, thiamine, and cyanocobalamin are reported to have antinociceptive effects in rodents (Bartoszyk and Wild, 1989). Pyridoxine was recognized in 1942 as causing severe weakness and pathological changes in peripheral nerves and dorsal root ganglia in dogs and rats (Antopol and Tarlov, 1942). Although the ease of producing such injury in several different species should have suggested that humans would also be vulnerable to pyridoxine neuropathy, it was not until 1983 that human neuropathy was reported (Schaumberg et al., 1983). Those first patients had taken large amounts of B6 for years, but subsequent reports indicated that as little as 200 mg/d might cause clinical symptoms (Parry and Bredesen, 1985).

Unfortunately, humans seem to be much more sensitive to this neurotoxic effect of pyridoxine than rodents (Xu et al., 1989). It is important to distinguish between clinical symptoms and asymptomatic abnormalities detected by electrical studies, because many patients are unaware of mild neuropathies. Coleman et al. (1985) treated children with Down's syndrome with high doses (25 mg/kg and up) of pyridoxine for years, starting in the first months of life. This treatment increased serum levels of PLP by 100–500-fold, confirming compliance with the treatment plan. Photosensitive skin lesions developed in 21 children after taking the drug for years; six children developed vomiting, which responded to reduced doses of the vitamin; and two developed evidence of peripheral neuropathy after many years on high doses. They improved when the vitamin was discontinued. Since nerve conduction studies were not routinely done on these children, and they were cognitively handicapped, it is unlikely that mild neuropathy would have been detected. Down's syndrome patients treated with pyridoxine for years did not differ in body size, head size, or measured intelligence from control children with Down's syndrome.

Animal studies show that pyridoxine doses without obvious clinical effect cause cell death (Phillips et al., 1978). Large doses of pyridoxine and PALP injected into the cerebral ventricles cause seizures in rodents (Weichert and Herbst, 1966). Perhaps more interesting is the report that ip injections of PALP increased susceptibility to audiogenic convulsions in a mouse strain (DBA/

2J) subject to audiogenic seizures (Norris et al., 1985). At the time of the increased seizure susceptibility, brain PALP was increased, as was brain aspartate and glutamate content; brain GABA concentration was decreased. A vitamin B6-deficient diet made these mice less susceptible to seizures. It is clear that pyridoxine is not a panacea for seizures.

Thiamine

Thiamine interactions with nerve, muscle, and brain are well described in older literature. Intravenous injections of large doses of thiamine produce death owing to respiratory failure in dogs (Smith et al., 1948), and direct application of thiamine to dog cortex causes seizures (Dias, 1947). No similar human experience has been reported, but anaphylactic reactions, some fatal, to iv thiamine are well known. The present ignorance of thiamine neurotoxicity is similar to the situation concerning pyridoxine before the 1983 report of Schaumberg et al.

Large doses of the vitamin had been known for years to have neurological effects in animals; the vitamin had been given to many humans in supraphysiological doses, and evidence of harm had not been found. It seems likely that very high doses of iv thiamine will be neurotoxic in some patients, particularly those with renal or hepatic failure. Unfortunately, large iv doses of thiamine are often given to emergency room patients (Wrenn et al., 1989), presumably because of the possibility that they may have Wernicke's encephalopathy, a common complication of alcoholism and malnutrition and one which can worsen if iv glucose is given without thiamine (Reuler, et al, 1985). Convulsions have been listed among the indications for iv thiamine, which leads to the suspicion that a second convulsion would not be considered a manifestation of vitamin toxicity even if that were the case. A recent report of more than 1000 patients receiving iv thiamine injections concluded that the practice was safe and rarely followed by significant complications (Wrenn et al., 1990).

Vitamin D

The hormonal form of vitamin D (1,25-dihydroxyvitamin D_3), produced by vitamin hydroxylation in the kidney, exerts its biological effects by binding to a nuclear receptor similar to those mediating the response to other steroid hormones (Norman et al., 1982). This receptor is found in a great variety of mammalian cells, including many without obvious special roles in calcium metabolism (Stumpf, 1988), and may be responsible for recently appreciated effects of 1,25-dihydroxyvitamin D_3 on cell growth and differentiation (Manolagas et al., 1987). The vitamin D receptor, together with steroid receptors, and those for thyroid hormone and RA, are probably all descended from a common ancestral gene (Evans, 1988).

Like retinoids, vitamin D_3 regulates PKC gene expression (Solomon et al., 1991). Whether vitamin D_3 receptors interact with hsp90 is currently unknown. No effects specific to the nervous system have been found to date, but one might predict that high circulating levels of this form of the vitamin will prove to have CNS effects (Luine et al., 1987) separate from the well known hypercalcemic effects of hypervitaminosis D, which primarily injures bone and kidney. Vitamin D_3 can prevent the development of experimental autoimmune encephalomyelitis in mice (Lemire and Archer, 1991) immunized with myelin proteins, but this may relate to its immunosuppressant effects rather than to a specific neurological action. Table 6 shows that cholecalciferol or vitamin D_3 stimulates ^{45}Ca fluxes in cultured cells of CNS origin. It should be noted that these in vitro effects of retinoids and cholecalciferol occur much more rapidly than the already known effects on gene expression that often take days to develop.

Vitamin E

The physiological function of vitamin E is poorly understood, as exemplified by differences of opinion concerning the symptoms vitamin E deficiency might cause. Roberts (1981) reported many patients with symptoms that he ascribed

to hypervitaminosis E because they were taking large doses of the vitamin. Obviously, they took the vitamin because of symptoms or fear of disease, and it is not possible to separate the causal factors in the patient group he describes. Vitamin E therapy has been advocated for many problems of premature infants (Phelps, 1988). Often, initial reports of benefit were followed by more extensive studies showing that it was not beneficial. Parenteral vitamin E was associated with a number of fatal reactions in infants (Balistreri et al., 1986). Vitamin E has been found to inhibit brain PKC in vitro, probably independent of its antioxidant effect (Mahoney and Azzi, 1988). In large doses, this vitamin might alter the subcellular distribution and enzyme activity of this very important kinase (assuming that permeability was not limiting).

Beneficial results of tocopherol treatment of strokes has been reported in experimental animals (Hara et al., 1990), and it has been given to Parkinsonian patients in a large trial of monoamine oxidase inhibitor therapy (Parkinson's study group, 1989). This study is still ongoing, but no large differences between vitamin E-treated and control patients were found in the first years of the study (Shoulson, personal communication), and no side effects have been recognized. Some patients taking large doses of vitamin E have developed weakness with histological and chemical evidence of muscle injury (Bardosi and Dickman, 1987). Vitamin E has been reported to improve seizure control when added to standard anticonvulsants in limited studies (Levy et al., 1990). Since the vitamin enters the CNS poorly, such an effect, if confirmed, would be most likely attributable to peripheral effects on drug metabolism.

Biotin and Other Vitamins

Biotin has been known to induce hepatic glucokinase activity in animals that are not vitamin-deficient. Recent work indicates that biotin injections produce a dramatic 19-fold increase in glucokinase mRNA within an hour in fasted, nondeficient rats (Chauhan and Dakshinamurti, 1991). A similar effect is produced by insulin. This effect is somewhat similar to effects of retinoids and vitamin D_3 on gene expression, but there is no present evidence of nuclear receptors, and the mechanism is unknown, although clearly not a coenzyme effect. A number of other biotin effects have been reported, some involving guanylate cyclase (Dakshinamurti and Chauhan, 1989). Table 6 shows that biotin increased ^{45}Ca influx into cultured C6 cells, like several other vitamins discussed. It may prove to be similar to retinoids and vitamin D_3, but little information is available about its possible CNS role. Space does not permit discussion of the possible roles of other vitamins in CNS function. Very little experimental data is available. Tissue culture provides an ideal medium for exploratory studies of the role of vitamins in CNS function but must of course, be supplemented by studies of intact organisms.

Discussion and Recommendations

Identification of vitamin neurotoxicity remains problematic. Many people take vitamins because they do not feel well. If their symptoms are the first sign of a disease that will produce neurological dysfunction, one might erroneously assume that the vitamin had caused the problem. Drug-induced disease is classically identified by induction of symptoms by drug exposure, recovery when the drug (vitamin) is discontinued, and relapse when the patient takes the vitamin again (drug challenge). Physicians rarely ask about vitamin intake and are slow to suspect that vitamins might be responsible for disease. Additionally, patients may refuse to accept second exposure to a drug suspected of having caused harm. Nowadays, physicians may be sued if the second exposure produces injury (i.e., proves that the vitamin was responsible).

Suspicion of possible vitamin toxicity is particularly unlikely when there is a long delay

between intake and development of symptoms or when the patient was seriously ill prior to taking the drug. For example, peripheral neuropathy and hearing loss are very common in the elderly, many of whom take vitamins in large doses. A group of California vitamin enthusiasts taking large doses of vitamin E had higher mortality rates than others with lesser vitamin E intake (Enstrom and Pauling, 1982). Was the vitamin responsible? There is no way to know since the groups were probably not comparable. How can we be sure that a drug or vitamin has caused symptoms or increased the incidence of a common problem? Improvement of symptoms with drug withdrawal is suggestive evidence, and when coupled with animal studies showing that the vitamin in question produces a similar neurotoxicity in animals, suspicion of human neurotoxicity is warranted.

This, however, will not settle all questions if the animal exposure requires large doses, intrathecal administration, and so forth. Neurotoxicity caused by large doses of pyridoxine was not discovered until more than 40 yr after the first animal studies (Schaumberg, 1983). This gap or delay suggests that many unwanted vitamin effects that are presently known only in animals will be found to occur in human patients. In addition, host factors or individual differences between patients, many of which are genetically determined, are surely important in determining vitamin tolerance. The higher the vitamin dose and the more rapidly it is administered, the greater the risk of harm. Administration of thiamine to alcoholics in emergency rooms is certainly justified, but the rate of administration and dosage may be excessive in some cases.

Intrathecal Vitamin Use

When trying to produce vitamin neurotoxicity, experimenters often inject vitamins directly into the brain or spinal fluid to circumvent permeability barriers. Given that all vitamins must be chemically reactive, such injection of vitamins into the human CNS seems distinctly hazardous. At present, injection of vitamins into the brain or spinal fluid is limited to two uncommon situations: treatment of inadvertent methotrexate overdosage and the aforementioned Japanese treatment for vasospasm associated with subarachnoid hemorrhage. Methotrexate (MTX) is a part of many cancer chemotherapeutic regimens and may produce neurotoxicity when administered systemically, usually only if combined with radiation therapy (Bleyer, 1981). It is often given intrathecally, and since the calculations for diluting it are moderately complex, there has been a continuing small incidence of inadvertent overdosage into the intrathecal space (Spiegel et al., 1984). Such patients are desperately ill and in recent years have often been treated with CSF lavage or ventriculocisternal perfusion. Such perfusion removes most of the drug and probably improves outcome.

Acting on the assumption that the toxic neurological effects of methotrexate (MTX) might be reversed by administration of physiological folates such as 5-formyltetrahydrofolate, some authors have advised intrathecal folates as part of the treatment (Ettinger, 1982). The case reports of such patients include convulsions (Spiegel et al., 1984), but it is unfortunately not possible to tell whether the folate "therapy" contributed to convulsions. However, our studies in normal rats show that addition of a reduced folate to FA or MTX increases rather than decreases the incidence of seizures (Table 8), exactly as would be predicted from the studies of Van Rijm et al. (1990). Furthermore, all the reduced folates cause seizures themselves (Van Rijm et al., 1990). Therefore, I believe that no folate is safe for intrathecal use. I have already discussed the use of ascorbate perfusion for subarachnoid hemorrhage and recommend that it be avoided for the two reasons that ascorbate is unlikely to reach the bleeding site and that it will probably reduce important surface proteins on neurons close to the infusion site (Levine, 1983).

Teratogenic Effects

Vitamins and other drugs frequently are better tolerated by adults and older children than

Table 8
Incidence of Convulsions After iv Folates in Rats

Folate and dosage	Incidence of convulsions
FA 10 μg	1/5
FA 50 μg	4/4
MTX 10 μg	0/3
MTX 50 μg	1/4
FA (10) + MTX (50)	4/7
THF (50) + MTX (50)	3/4

Rats (150–200 g males) were briefly anesthetized with ether, received stereotactic injections of artificial CSF containing 1 mg/mL sodium ascorbate to protect folates from oxidation, and were observed for 90 min. The number given is the ratio of rats observed to convulse divided by the number injected. No rats receiving control injections of artificial CSF convulsed. FA, folic acid; MTX, methotrexate; and THF, tetrahydrofolate.

infants and fetuses. Vitamins have been advocated to prevent neural tube defects, a serious congenital abnormality of the nervous system for which genetic predisposition exists in some families (Hall et al., 1988). Several studies indicate reduced incidence of neural tube defects in pregnancies after periconceptual vitamin supplementation (Mills et al., 1989). It may be that vitamin deficiency increases the risk of neural tube defect and that hypervitaminosis causes other congenital malformations. Since vitamins A and D have special effects on gene expression, excessive intake of these vitamins seems more likely to produce congenital malformations than other vitamins.

Vitamin A

Vitamin A excess and deficiency are both neurotoxic in several ways. Large doses are well known to produce anencephaly in rats. Although vitamin A neurotoxicity has been known since 1954, and large doses of retinoids are clearly teratogenic in animals (Cohlan, 1953), maternal ingestion of vitamin A itself in ordinary doses is not associated with increased risk of fetal malformation (Werler et al., 1990). Because of the tendency for vitamin A to accumulate in the tissues, mothers who take large amounts prior to conception may be exposing the fetus to vitamin A injury even if they reduce vitamin intake upon becoming pregnant (Hathcock et al., 1990). The teratogenic potency of retinoids varies between species. Table 9, modified from Teratology Society (1987), shows that primates are not always the best guide to human teratogenic risk.

Ascorbic Acid

Little is known about ascorbate's teratogenic potential.

Folates

Both folate deficiency and excessive maternal folate intake reduce litter size and brain in offspring of pregnant rodents (Middaugh et al., 1976).

Pyridoxine

High doses of pyridoxine are teratogenic in rodents and other laboratory animals. The drug Bendectin™, a combination of pyridoxine and doylamine, was formerly used extensively as an antinauseant during pregnancy. During the 1970s, more than 10% of pregnant women in the US may have taken this drug. It has been withdrawn from the market after lawsuits and other controversies alleging that it increased the risk of congenital malformations in humans. No controlled studies were ever done, and the rodent data suggest that it produces teratogenic effects only at high doses likely to have effects on maternal health as well (Tyl et al., 1988). The threshold dose for human teratogenic effect is unknown, but doses greater than the RDA are unwise.

Vitamin D

No evidence of vitamin D teratogenicity without effects on maternal health is known. The RDA should not be exceeded in pregnancy.

Discussion

This review has stressed three basic ideas: Vitamins are by definition reactive, and supranormal vitamin concentrations are likely to produce noncoenzyme effects, some of which are injurious. These are more likely to occur in such per-

Table 9
Teratogenic Doses of Retinoids in Different Species

Species	Retinol	Tretinoin	Etrinate	Isotretinoin
Human	unknown	unknown	0.2	0.4
Monkey	unknown	7.5	5.0	5.0
Rat	50	0.4	2.0	150.0
Mouse	75	4.0	4.0	100.0
Hamster	15	12.5	2.8	25.0

These are the lowest known doses of each retinoid reported to cause teratogenic effects, in mg/kg/day, in each species. However, different strains within species vary in sensitivity. Modified from the Teratology Society (1987).

ipheral organs as the liver, which is not protected by the blood-brain barrier and has a higher concentration of all vitamins than brain. Patients with reduced intracellular vitamin-binding proteins (cf Boyland and Gudas, 1991) or mutant vitamin metabolizing enzymes may be injured by ordinary doses of vitamins. Liver disease may impair the production of vitamin-binding proteins. Second, several vitamins produce convulsions when given into the CNS at high dosage. These same vitamins probably have similar effects in humans. In the case of folates, Ch'ien's patient with two folate-provoked convulsions represents such an occurrence (Ch'ien et al., 1975). The third point is that long-term controlled studies of the effects of high doses of vitamins on subtle neurological functions such as attention, memory, and "intelligence" are needed. Such studies might show that ordinary doses of vitamins improve some cerebral functions in certain patients or age groups but impair other cerebral or peripheral functions in other humans. The author has provided new data showing that microgram amounts of several vitamins alter calcium fluxes in cultured cells and stimulate calcium-dependent enzymes, and evidence that all folates produce convulsions when injected into the lateral ventricles of rats. These data confirm the potential effects, desirable and undesirable, of vitamins on CNS function.

Neundorfer (1980) reviewed the neurotoxic effects of vitamin deficiency and vitamin excess and noted that hypervitaminosis A was the only established human vitamin neurotoxicity. Pyridoxine qualifies for this categorization today, and at least one case of human folate neurotoxicity exists (Ch'ien et al., 1975). Considering the existing animal data, we predict that folate and thiamine will soon be admitted to the group of neurotoxic vitamins. Whether ascorbate and vitamin D prove to be neurotrophic, neurotoxic, or both, like amyloid β-protein, remains unclear. Ascorbate modification of protein structure and function, particularly of NMDA receptors, may prove important because of its very high concentration in the brain.

In summary, vitamins have effects beyond their traditional coenzyme roles. These additional effects may be beneficial or harmful to CNS function, depending on the circumstances. Our view of vitamin A functions has drastically changed, and that of vitamin D may follow a similar course (Dabek, 1990). Permeability barriers restrict vitamin entry to the CNS, and most cases of vitamin neurotoxicity are explained by abnormalities in permeability (or direct injection of vitamins into brain or CSF), abnormalities of vitamin-binding proteins, or megavitamin intake. Because vitamins are profitable, the public will continue to be exposed to promotions for high-dose vitamin intake. In much of the world, malnutrition and vitamin deficiencies are common health problems. Vitamin toxicity is a public health problem only for the affluent. Symptoms of vitamin neurotoxicity may be subtle and develop only after prolonged exposure. The genetic heterogeneity of the human race suggests that risk-benefit calculations for high-dose vitamin intake will have

to be individualized. Because contaminants produced in vitamin manufacture may have biological effects, quality control of vitamin production should be improved and consumption of natural vitamins (i.e., food) should be preferred to use of synthetic supplements.

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