THE EFFECT OF NUTRITION ON CHEMICAL TOXICITY

D.V. Parke[1], M.S.I. Dhami[2] and M. Afzal[3]

[1]School of Biological Sciences, University of Surrey, Guildford, UK
[2]Nuclear Sciences, San Jose State University,
San Jose, CA 95192, USA, [3]Department of Biochemistry
Faculty of Science, Kuwait University, Kuwait

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SUMMARY

The toxicity of chemicals, and of reactive oxygen species (ROS), are both affected by nutrition and diet. Calorific excess (continuous feeding), or deficiency (fasting), may increase production of ROS, which are also formed by interaction of toxic chemicals with cytochromes P450 (CYP2E or futile cycling). Both ROS (GSH reductase and peroxidase) and toxic chemicals (S-transferases) are detoxified by GSH enzymes; ROS are scavenged by a system comprising GSH, ascorbic acid and tocopherols, which may be regenerated by NADPH. Dietary protein is necessary for GSH or enzyme replacement, lipids are required for polyunsaturated fatty acids (PUFAs) and prostanoid biosynthesis, lipotropes and phospholipids for synthesis of endoplasmic reticulum, and folate is needed for dug metabolizing activity. Among required minerals, Se is necessary as the essential component of the antioxidant enzyme, glutathione peroxidase. Other dietary factors considered are the natural toxicants, gossypol, lathyrogens, glucosinolates, and saponins, and toxicants from food spoilage, food intoxication and food processing.

1. INTRODUCTION

Man and other animals have evolved in a hostile environment of toxic chemicals and reactive oxygen species (ROS) which would quickly prove lethal were it not for the biological defense systems that have developed to protect the organism from the damaging effects. The biological defense system against reactive oxygen, *the antioxidant system,* evolved first, to protect the cell against the destructive effects of ROS (Figure 1). Then with the emergence of multicellular flora and fauna, the development of toxic phytoallexins by plants to deter animal predators, and the consequent development of some protection against these toxins by the animal species, a chemical defense system of *detoxication* (Figure 1) progressively evolved, over some 300 million years of "plant-animal warfare" /20/. These two biological defense systems have a common factor in that glutathione (GSH) is an essential component of both systems (Figure 1) with the consequence that some species, e.g. rodents, which as small mammals use GSH extensively for detoxication of ROS, are increasingly vulnerable to the toxicity of chemicals /70/. Furthermore, it is now recognized that much chemical toxicity involves molecular mechanisms which are mediated by ROS,

Fig. 1: Glutathione - a critical component of the antioxidant and detoxication defense systems.
GPX = glutathione peroxidase; PHGPX = phospholipid hyhdroperoxide glutathione peroxidase.

e.g. redox cycling of the quinoneimine metabolite of paracetamol /17/, so that protection from environmental chemicals frequently requires the functioning of both systems.

As the individual components of these two interrelated biological defense systems are largely expended in their protective roles, and require frequent dietary replenishment, the efficiency of both the detoxication and the antioxidant systems is dependent largely on the adequacy of the diet. This is a factor which has been largely overlooked, so that the importance of nutrition and diet in the manifestation of chemical toxicity has been mostly ignored. Diet and nutrition are well-known to have profound effects on the pharmacological and pathological response of laboratory animals to drugs and environmental chemicals /68-70/, overnight fasting is known to evoke major changes, both qualitative and quantitative, in the cytochromes P450 /52/, and the *ad libitum* feeding of rodents markedly increases the carcinogenesis of chemicals /14,75,90/. Yet these major variables are completely disregarded when animal pharmacokinetic or pharmacodynamic studies are undertaken, or long-term animal toxicity and carcinogenicity study protocols are designed, and most scientific papers in the fields of toxicology and drug metabolism contain no information regarding diet even though this can evoke greater variations than most of the environmental factors that are traditionally listed. Indeed, it was the realization that relatively simple dietary changes were sufficient to transform a negative rodent carcinogenicity study into a positive one, and vice versa, and that fasting could change the whole pattern of drug metabolism, which convinced the authors of the need to apprise fellow toxicologists of the importance of diet in the prevention and manifestation of chemical toxicity and of the many pitfalls that abound when this aspect is ignored in toxicological evaluations.

2. BIOLOGICAL DEFENSE AGAINST OXIDATIVE STRESS AND CHEMICAL TOXICITY

In all biological systems, reactive oxygen species (ROS) are being continuously generated by the one-electron reduction of molecular oxygen, often the result of imperfect electron conductivity in biological membranes, or the consequence of redox cycling. The ROS comprise the following: singlet oxygen ($^1O_2$), superoxide anion/radical ($O_2^{\cdot -}$), peroxide (O_2^{2-}), and hydroxyl radical ($\cdot OH$), all of which are toxic,

resulting in oxidative stress and depletion of glutathione, lipid peroxidation and membrane destruction, DNA damage and mutations, malignancy and cell death (Table 1; Figure 2) /23,24,69/.

The biological *antioxidant defense system* is an integrated array of enzymes, antioxidants, and radical scavengers, including glutathione (GSH), glutathione reductase (GSSG reductase), the glutathione S-transferase family of enzymes /10,32/, glutathione peroxidase (GPX), phospholipid hydroperoxide glutathione peroxidase (PHGPX) /91/, superoxide dismutase (SOD) and catalase, together with the antioxidant, radical-trapping molecules, ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids /39/, ubiquinone and bilirubin. However, recent studies have indicated that all of these antioxidant, radical-trapping agents are related to the intracellular levels of reduced

TABLE 1

Some biological origins of reactive oxygen species (ROS)

Origin of ROS	**Reference**
Ionizing or UV radiation	
Leucocyte activation	Biemond *et al.* /1/
Electron leagkage from mitochonodrial cytochrome chain	Sohal *et al.* /83/
Transoxygenation - associated with conversion of PGG_2 to PGH_2 and HPETE to HETE in eicosanoid synthesis	Krauss and Eling /37/
Non-haem iron components	
Cytochrome P4502EI	Ekstron and Ingleman-Sundberg /13/
Futile cycling of activated microsomal cytochromes P450	
Redox cycling of xenobiotic quinones	Powis and Appel /73/
Carbon-centered radicals of xenobiotics	

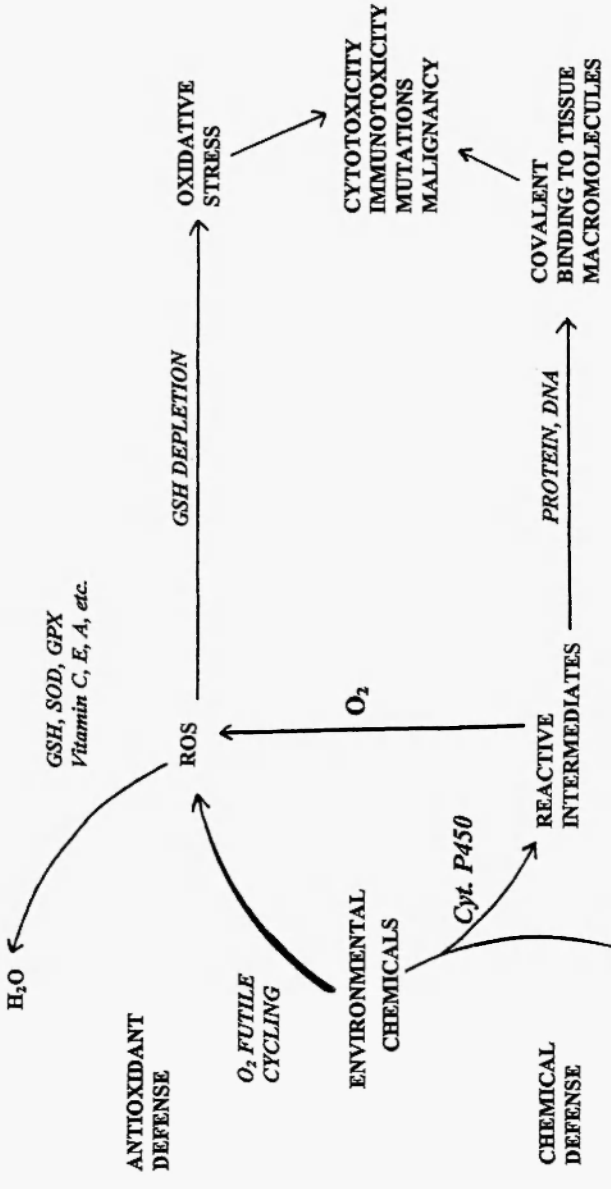

Fig. 2: Roles of the detoxication and antioxidant defense systems in protection against chemical toxicity.
SOD = superoxide dismutase; GPX = glutathione peroxidase; ROS = reactive oxygen species;
GSH = glutathione; UDPGA - uridine diphosphoglucuronic acid; PAPS = phosphoadenosine-phosphosulfate.

glutathione, which appears to be the ultimate protection against oxidative stress /53/. This again emphasizes the importance of conserving GSH for antioxidant defense, rather than using it indiscriminately to conjugate epoxides as rodents do /70/, and the need for adequate nutrition to supply plentiful NADPH for maintaining the level of intracellular reduced glutathione via GSSG reductase.

The chemical defense system of detoxication, which protects the animal organism against the accumulation and adverse effects of toxic chemicals, depends on oxidative metabolism (Phase I reactions) and conjugation (Phase 2 reactions), catalyzed mostly by the microsomal oxidases (cytochromes P450) and the many glucuronyl-, sulfo-glutathionyl- and other transferases of the liver, kidney, gastrointestinal tract, and other tissues (Figure 1). However, the metabolism of a drug or xenobiotic chemical does not always result in detoxication; microsomal oxidation can result in the production of a reactive intermediate which, because of conformationally-hindered oxygenation, is not readily conjugated and as a highly reactive electrophile interacts with protein, DNA, etc. to form neoantigens and DNA adducts, resulting in toxicity and carcinogenicity (Figure 2). Similarly, conjugations can result in toxicity as well as detoxication, for example protein acylation by metastable acyl glucuronides /84/, and the β-lyase metabolism of glutathione conjugates to toxic thiols. Toxic chemicals may often manifest their toxicity by molecular mechanisms involving ROS, for example, by (i) depletion of intracellular GSH, (ii) dehalogenation by cytochromes P450 to carbon-centered radicals, (iii) generation of ROS from induction of cytochromes P450 and futile cycling, from the redox cycling of quinone metabolites, and from the formation of neoantigens and the consequent immunotoxicity. Hence, the two biological defense systems against ROS (antioxidant defense) and toxic chemicals (detoxication) are intimately related, and in considering the nutritional needs of the various detoxication processes to prevent or minimize chemical toxicity, the nutritional requirements for protection against ROS toxicity and oxidative stress must also be included (Figure 2).

3. UNRESTRICTED FEEDING AND CHEMICAL TOXICITY

Rodents on calorie restricted diets have a much lower incidence of spontaneous and chemically-induced tumors than animals fed *ad libitum* (Table 2) /26/. Indeed, calorie restricted rats were completely refractive to 7,12-dimethbenz(*a*)anthracene-induced mammary tumors,

TABLE 2

The effects of calorie restriction on the incidence of tumorigenesis in rodents

Tumor type	Species	Initiator	Incidence		References
			Unrestricted Feeding	Calorie Restriction	
Mammary	Mice	Spontaneous	40	2	Tannenbaum /88/
Lung	Mice	Spontaneous	50	30	Larsen & Heston /45/
Leukaemia	Mice	Spontaneous	65	10	Saxton *et al.* /80/
Skin	Mice	Benzo(*a*)pyrene	65	22	Tannenbaum /89/
Skin	Mice	3-Methylcholanthrene			
		Low fat	54	0	Lavik & Baumann /46/
		High fat	66	28	Lavik & Baumann /46/
Colon	Rats	Azoxymethane			
		Low fat	56	41	Kumar *et al.* /41/
		High fat	85	56	Kumar *et al.* /41/
Leukaemia	Mice	γ-Irradiation	50	4	Gross /22/
Liver	Mice	Diethylnitrosamine	100	0	Lagopoulos & Stadler /43/
Liver	Rats	Aflatoxin B_1	77	32	Newberne & Rogers /64/

even though such diets had a high fat content /40/. Calorie restriction in rodents alters energy metabolism through changes in key enzymes of glycolysis, gluconeogenesis and lipid metabolism (Table 3). Rodents fed unrestricted diets exhibit higher oxygen consumption, decreased insulin binding, and modified gene expression, compared with animals on restricted diets /26/. Drug metabolizing enzyme activites are different in rodents fed *ad libitum* diets from those on restricted calorie intake (Table 3); *ad libitum* diets decrease the activities of several

TABLE 3

Effect of calorie restriction on activities of enzymes of intermediary metabolism and drug metabolism in rodents

	Ad lib Feeding	Calorie Restriction	% Change
Glucose 6-phosphatase‡	0.22	0.33	+50
Pyruvate kinase†	6620	3410	-50
Lactate dehydrogenase†	1460	770	-50
Glycerol kinase†	2590	1170	-50
Fatty acid synthetase†	1790	1520	N.S.
Testosterone 16α-hydroxylase*	2.67	1.71	-36
Testosterone 6β-hydroxylase*	0.12	0.09	-25
Testosterone17-oxidase*	0.87	1.79	+250
4-Nitrophenol hydroxylase*	0.43	0.91	+110
7-Ethoxyresorufin O-deethylase‡	0.04	0.045	N.S.
Aminopyrine N-demethylase*	3.50	3.20	N.S.
Glutathione S-transferase* (1,2-dichloro-4 nitrobenzene)	27.6	39.2	+42
UDP-Glucuronyltransferase* (2-aminophenol)	3.30	5.40	+120
N-Acetyltransferase*	2.10	4.60	+120

*nmol/min per mg liver microsomal protein
†units/l
‡μmol/min per mg liver microsomal protein
[Data taken from Feuers *et al.* /16/; Leakey *et al.* /47,48/; and Hart *et al.* /26/.]

Phase 2 enzymes, including the UDP-glucuronyltransferases, glutathione S-transferase and N-acetyltransferase, which would explain the increased incidence of chemical carcinogenesis. In rodents fed unrestricted diets, there is progressive age-related degradation of the antioxidant defense enzymes, catalase and superoxide dismutase, which would lead to an increase in ROS-mediated toxicity, and hence carcinogenicity; calorie restriction inhibits these age-associated enzyme degradations /26/. Unrestricted feeding of rats also decreased the total hepatic cytochrome P450, increased aflatoxin B_1 activation, increased aflatoxin binding to DNA and decreased the *in vivo* detoxication of this carcinogen. DNA methylation in mice was decreased and oncogene expression was increased, by feeding unrestricted diets /26/. Thus, the unrestricted feeding of rodents results in fundamental metabolic changes that lead to increased oxygen consumption, increased rates of degradation of antioxidant defense enzymes, decreased activities of conjugase enzymes, decreased total liver cytochrome P450 with changes in the pattern of isozymes, enhanced carcinogen activation and decreased detoxication, which together with the increased oncogene expression leads to a significant increase in chemical toxicity and tumorigenesis.

4. FASTING AND CHEMICAL TOXICITY

It has long been the practice in *in vitro* drug metabolism studies to starve animals overnight, supposedly to decrease the liver glycogen which interferes with the preparation of microsomal enzyme fractions, and in pharmacokinetic studies to carry out the gastric intubation of drugs and chemicals on fasted animals so that the presence of food in the stomach would not impede gastric absorption. Similarly, elective surgical patients are fasted overnight, ostensibly to prevent regurgitation of fluids into the airways. Such animals and patients are considered to be physiologically normal and little or no consideration has been paid to the effects of fasting on the mechanisms of drug metabolism, drug pharmacodynamics, or chemical toxicity.

In 1979, Pessayre drew attention to the marked difference between the hepatotoxicity of the classic liver toxin, bromobenzene, in fed and fasted rates /72/. Using levels of serum glutamate/pyruvate transminase as an index of hepatic damage, it was shown that rats fasted for 24 hours manifested hepatotoxicity at an oral dose of 125 mg/kg of bromobenzene, whereas fed rats showed no toxicity even at six times

this dose level (750 mg/kg). This was attributed to the detoxication of bromobenzene by liver glutathione, as previously shown by Stekol /85/, the levels of which are greatly depleted by 24 hour fasting /53/. Fasting is known to induce the activity of cytochrome P4502E1 in the liver and kidney of rats /29/, and Liu *et al.* /54/ further showed that the traditional practice of overnight fasting and ether anesthesia in experimental animal procedures, and in the preparation of tissue subcellular fractions, resulted in glutathione depletion, induction of CYP2E1, generation of ROS, oxidative stress, lipid peroxidation, and a dramatic loss of other isoforms of cytochrome P450 /52/. These pathological effects were attributed both to the 24 hour fasting and to the toxic effects of the ether anesthesia, the effects being additive /54/.

The microsomal UDP-glucuronosyl transferases /2/, like the cytochromes P450, are integral components of the endoplasmic reticulum, and lipid peroxidation of these membranes, induced by fasting or exposure to substrates of CYP2E1, results in marked losses in both groups of enzymes leading to decreased glucuronide conjugation and decreased detoxification /4/.

5. PROTEIN

Apart from the dietary needs of protein for growth, tissue repair, renewal of epithelia and blood cells, protein is also needed for the replenishment of the numerous enzymes of the antioxidant defense system (superoxide dismutase, catalase, glutathione reductase, peroxidase, etc.), and of the chemical detoxication system (the P450 superfamily, UDPGA transferases, GSH S-transferases, etc.). Dietary protein is also needed for the biosynthesis of glutathione, the intracellular redox buffer, which has vital roles in protecting the cell against the toxic effect of ROS and the consequences of oxidative stress, and in detoxicating environmental chemicals and their reactive metabolites (epoxides) by conjugation to give excretable mercapturic acids. The amino acids, glycine, glutamine, cysteine and taurine, are also involved in the conjugation of drugs, environmental chemicals and their metabolites, and the sulfur amino acids (cysteine, cystine, methionine) are oxidized to yield sulfate (i.e. PAPS, phosphadenosine phosphosulfate) for the conjugation and detoxication of phenols (e.g. paracetamol) and other chemicals.

Hepatic microsomal oxidase activities increase with increase in dietary protein up to a protein content of 30% /34/. In general, higher microsomal oxidase activity means increased cytochrome P450 activities and increased detoxication, so that high protein diets result in lower toxicity of chemicals, as may be seen from the acute oral toxicities of a number of pesticides in rats fed on high and low protein diets (Table 4) /3/. However, the converse may occur with some carcinogens that are activated by oxidative metabolism. High protein diets enhance oxidative drug metabolism in humans, and accelerate the metabolism of antipyrine, theophylline, propranolol, and other drugs; a change of parenteral nutrition of healthy volunteers from glucose to amino acids increased the clearance of aminopyrine by 20% /67/. Methionine is important for effective utilization of selenium and hence for GSH peroxidase activity and the detoxication of lipid peroxides /87/; methionine deficiency also decreased liver GSH and increased the hepatotoxicity of paracetamol in mice /59/ and in rats /74/.

TABLE 4

Acute oral toxicities for pesticides administered to rats on low and high protein diets

Pesticide	LD_{50} (mg/kg body wt)		Ratio of LD_{50} values on normal/low
	on low protein diet	on normal protein diet	
Chlordane	135±30	265±45	2.0
Lindane	95±35	185±16	2.0
Malathion	600±140	1400±100	2.3
DDT	165±35	480±15	2.9
Endosulfan	24±10	100±16	4.2
Carbaryl	89±11	575±51	6.5
Parathion	4.9±1.3	37±5	7.6
Captan	480±110	12600±2100	26

Data taken from Boyd and Taylor /3/.

6. LIPIDS

Lipids are usually regarded as a fairly homogeneous group of nutrients, an excellent source of energy, and valuable in the synthesis of biological membranes. This naive concept has been largely responsible for most of the misconceptions about lipids and toxicity which have found their way into medical literature in the past few decades, for lipids such as phospholipids and polyunsaturated fatty acids (PUFAs) are essential nutrients, vital to the synthesis and functioning of biological membranes, and to the biosynthesis of the prostaglandins and various other prostanoids.

Diets containing different sources of protein with high fat content promote spontaneous incidence of cancer and potentiate the tumorigenicity of carcinogenic chemicals (Table 5), although Newberne has shown this may be associated with low dietary intake of lipotropes /62/. Dietary lipotropes, including choline, methionine, glycine, folate, vitamin B_{12}, pyridoxal, polyunsaturated fatty acids and phosphate, are required for the synthesis of phospholipids and biological membranes, essential for microsomal metabolism and the detoxication of xenobiotic chemicals and carcinogens. Phosphatidylcholine is an essential component of the microsomal oxidase system, and reconstituted solubilized enzymes, the cytochromes P450 and NADPH-cytochrome P450 reductase, require phosphatidylcholine for full enzymatic activity /55/. A choline-deficient diet, in the absence of any carcinogen or toxic chemicals, results in lipid peroxidation and hepatotoxicity in rats within days and induces hepatocellular carcinoma in more than 50% of the animals within two years /62,79/. Dietary deficiencies in the lipotropes, choline and methionine, enhance the carcinogenicity of aflatoxin, 2-acetamidofluorescein and ethionine /63/. The microsomal oxidase system is affected both qualitatively and quantitatively by dietary fat /94/ and although PUFAs are essential for eicosanoid, phospholipid, and membrane biosynthesis, increased dietary intake of PUFAs increases the susceptibility of biological membranes to lipid peroxidation, exposing the cytochromes P450 to ROS-mediated attack and consequent loss of enzymatic activity /92/. Dietary fiber also plays a role, and dietary fat and fiber affect N-methyl-N-nitro-N-nitrosoguanidine-induced colon cancer in a complex interactive manner; fat had little effect when dietary fiber was high but increased tumor incidence when fiber was low /81/.

TABLE 5

Liver tumor incidence in rats fed different sources of protein

Protein source (%)						Tumors[3]	
	Peanut Meal						
Dried beef[1]	Unextracted	Alcohol extracted[2]	Casein	Choline content added (%)	Days on diet (mean)	Liver	Kidney
31.7	-----	-----	-----	0.3	448	0/5 (0)	0/5 (0)
31.7	-----	-----	-----	0.0	448	0/10[4] (0)	0/10 (0)
7.9	-----	33.3	-----	0.0	432	6/10[4] (60%)	0/10 (0)
7.9	-----	33.3	-----	0.3	481	1/5 (20%)	0/5 (0)
-----	-----	34.3	-----	0.3	410	1/5 (20%)	0/5 (0)
6.0	-----	25.0	-----	0.0	558	2/9[4] (22%)	0/9 (0)
-----	33.3	-----	7.9	0.0	470	15/15 (100%)	1/15 (7%)
-----	33.3	-----	7.9	0.6	480	9/10 (90%)	2/10 (20%)
7.9	33.3	-----	-----	0.3	424	8/10 (80%)	2/10 (20%)
-----	33.3	-----	7.9	0.3	334	4/8 (50%)	1/8 (12%)

[1]All diets contained 20% fat from beef tallow; [2]Extracted 72 hours with methanol; [3]Abridged from Salmon and Newberne /77/; [4]Livers were cirrhotic. (Data taken from Newberne /62/.)

7. VITAMINS

Ascorbic acid (vitamin C) is essential for the microsomal oxygenation of many xenobiotics, and for the detoxication of lipophilic toxic chemicals, firstly by facilitating the introduction of a polar oxygen function by the cytochromes P450, and secondly by facilitating the detoxication and elimination of these polar metabolites by UDPGA-mediated conjugation to glucuronides /19/ (Figure 2). It decreases chemical toxicity by decreasing the covalent binding of reactive intermediates, reducing quinones, eliminating free radical metabolites, and inhibiting the formation of toxic nitrosamines from the nitrosation of secondary amines /19/. Ascorbic acid deficiency increases the fluidity of the microsomal membranes, thereby decreasing microsomal oxidase activity and the activity of the microsomal UDPGA transferases /61/; as the latter are considered to be among the most important detoxication enzymes in mammals, the fundamental role of ascorbic acid in xenobiotic metabolism and chemical toxicity is clearly evident.

Riboflavin is an essential component (FAD and FMN) of the NADPH-cytochrome P450 reductase; FAD is the electron acceptor from NADPH and FMN is the electron donor to P450. In riboflavin deficiency, FAD is in excess over FMN resulting in an abnormal P450 reductase /25/ that might result in electron leakage (uncoupling) and ROS generation. Riboflavin also potentiates nitro- and azo-reductase activities and azo dye induced cancer in rats has been shown to be related to the riboflavin content of the diet and liver /95/.

Folate is also needed for drug metabolism and chemical detoxication, especially when there is induction of the hepatic microsomal oxidases. Administration of poor folate diets to institutionalized epileptics on phenobarbitone and diphenylhydantoin led to progressive depletion of folate, loss of drug metabolizing activity, loss of enzyme induction, hyperchromic anemia and teratogenic effects. Subsequent studies in rats have confirmed these findings that folate is essential for the increased turnover of the drug metabolizing enzymes that occurs during chronic drug administration /42/.

In contrast to ascorbate, riboflavin and folate, **thiamine** deficiency actually increases cytochrome P450 activity, and the metabolism of aminopyrine, ethylmorphine, and many other xenobiotic chemicals; deficiency of this vitamin specifically increases cytochrome P4502E1 activity, which induces ROS and is concerned in the ROS-mediated metabolic oxygenation of alcohol, benzene, and other chemicals /98/.

Vitamin E, the tocopherols, are important biological antioxidants, scavenging free radicals, quenching the reactivity of singlet oxygen and thereby protecting the microsomal membranes against lipid peroxidation, loss of cytochrome P450 and drug metabolizing activity /33,58/.

Dietary **vitamin A** and retinoids have a protective effect against chemical carcinogens, deficiencies increasing the binding of benzo(*a*)-pyrene (BP) metabolites to DNA, while supplementation during and after administration of BP to hamsters decreased the incidence of respiratory tumors by 90% /63/ (Table 6). Retinol also decreases the mutagenicity of food pyrolysis heterocyclic amines, the aminoimido-azaarenes, by inhibiting their metabolic activation by cytochrome P451A2 /30/. Similarly, carotenoids have been shown to inhibit the mutagenicity of aflatoxin B_1, independent of any conversion to retinol /27/.

8. MINERALS

Although iron is essential for the biosynthesis of haem, synthesis of cytochromes P450, and microsomal oxidase activity, iron deficiency resulting in anemia does not result in depletion of cytochromes P450.

TABLE 6

Effect of vitamin A on benzo*(a)*pyrene (BP)-induced lung cancer in hamsters

Diet	Malignant tumors of respiratory tract	
	Number	%
Low vitamin A (0.3 μg retinyl acetate/g)	102/127	80
High vitamin A (30 μg retinyl acetate/g)	40/88	46
Optimum vitamin A (2 μg retinyl acetate/g)	46/89	52
plus cis-RA* during BP dosage	38/83	46
plus cis-RA* after BP dosage	11/84	13
plus cis-RA* before and after BP	4/91	4

*cis-retinoic acid (Data taken from Newberne and McConnell /63/.)

Conversely, excess iron may enhance the production of ROS /7/ resulting in lipid peroxidation, destruction of cytochromes P450 and loss of hepatic microsomal oxidase activity /96/. Dietary supplementation of iron increased the promotion of 1,2-dimethylhydrazine-induced colorectal cancer in rats, which was reversed by phytic acid present in dietary fiber, probably due to its chelation of the iron and the consequent inhibition of iron-induced generation of ROS /60/.

Selenium is an essential component of glutathione peroxidase (GPX) and phospholipid hydroperoxide glutathione peroxidase (PHGPX) which protect biological membranes against lipid peroxidation and damage by ROS. Se-deficient diets consequently increase lipid peroxidation, and reduce the levels of cytochromes P450, enzyme induction, the detoxication of chemicals and increase chemical-induced carcinogenesis /31,97/. Selenium and selenate are widely distributed in liver, kidney, muscles, gastrointestinal tract and blood /5/. Selenite and selenate are metabolized to trimethyl selenium ion which is the principal excretory product in the urine. The toxicity of selenium is modified by its interaction with sulfate, methionine, cystine, mercury, lead and copper /28/. Acute and chronic human toxicosis has been reported from industrial and other accidental exposures /9/, and from the ingestion of selenium-containing foodstuffs. Other substances can alter the toxicity of selenium, including sulfate, methionine, cysteine, various heavy metals, arsenic, vitamins C and E /93/. Epidemiological studies in children have shown an increase in the rate of cavities during the development of teeth when high levels of selenium are ingested /6/. Limited studies indicate a role against human cancer. Fan and Kizer /15/ have extensively reviewed the nutritional, toxicologic and clinical aspects of selenium because of growing public interest in selenium as a dietary supplement and the incidences of environmental contamination.

Magnesium increases liver microsomal cytochrome P450 content, together with NADPH-cytochrome P450 reductase, and microsomal oxidase activities, possibly by its protection of glutathione from oxidative damage.

9. OTHER DIETARY FACTORS

Alcohol is essentially a source of energy devoid of any other valuable nutrients, which, in excess, results in the induction of cytochrome P4502E1, promoting ROS generation, oxidative stress and tissue damage /8/. Cytochrome P4502E1 has a high potential to

destroy cytochrome P450 activity, by lipid peroxidation, and to activate toxic chemicals by its generation of ROS; the ROS are themselves highly cytotoxic and carcinogenic. This explains the known inhibition of drug metabolism and detoxication by the administration of alcohol, and its potential to deplete vitamin E and other antioxidants /35/ and to cause gastritis, hepatotoxicity and carcinogenesis /50/.

The flavonoids, which occur widely in fruits and vegetables, protect against hepatotoxicity, probably by preventing lipid peroxidation /11/. The monoterpenoids, d-limonene and menthol, have also been shown to be anticarcinogenic /76/.

During the past decade, a new class of toxic chemicals, heterocyclic amines, derived from frying, grilling or roasting meats, fish and other foods (food pyrolysis), have been shown to be highly potent mutagens and potential carcinogens /86/, activated by N-hydroxylation involving cytochrome P4501A2 /71/. The extent of their formation is dependent on the cooking temperature and method, and fried or flame-grilled foods are 10-fold more mutagenic than boiled or baked foods /12/.

10. FOOD TOXICANTS

Modern technological advances in food production, processing, distribution and marketing have also resulted in generating a great awareness of the problems of food safety (Figure 3). A comprehensive list of some common food additives for preservation and enhanced shelf-life is given in Table 7. Naturally occurring food toxicants, contaminants and food additives are receiving increasing attention due to concerns over public health. Many factors influence the presence of 'toxicants' in food from the time of its production, through processing, storage, marketing and consumption /18/.

Human food consists of a variety of materials derived from plants and animals. In addition to the presence of nutrients essential for proper growth and health, these foods also contain substances which possess toxicological properties. When consumed, they may cause conditions from minor discomfort to impaired nutrient availability, and onset of distinct disease states such as cancer. Some important types of toxicants present in plants include the following:

10.1 Enzyme inhibitors

Salunke and Wu /78/ have extensively reviewed most of the

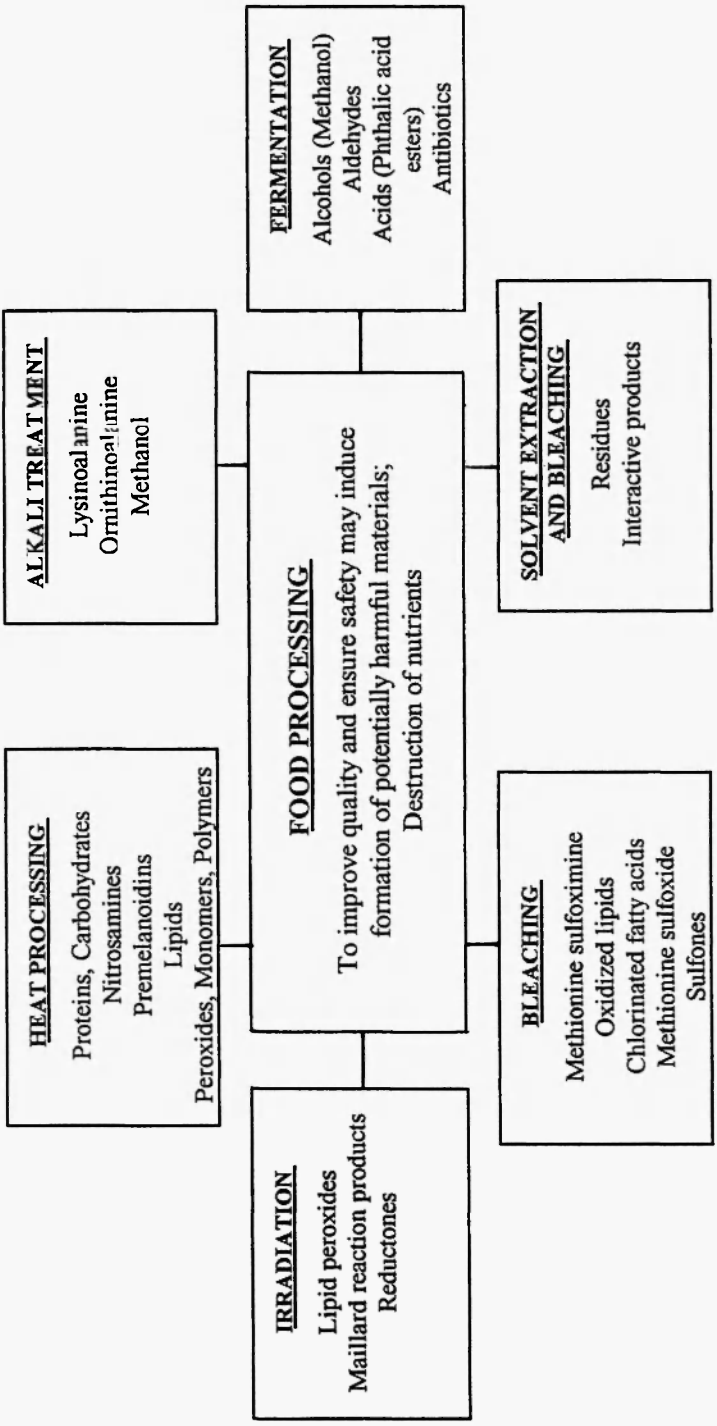

Fig 3: Common processes foods undergo which may result in the production of toxic compounds.

TABLE 7

Some common food additives

CLASS	FUNCTION	SOME EXAMPLES OF SPECIFIC ADDITIVES	FOODS IN WHICH THESE ADDITIVES ARE USED
ANTICAKING AGENTS	keep powders free-running	magnesium carbonate	icing sugar
BLEACHING AND MATURING AGENTS	hasten the natural process of whitening and maturing of wheat flour	chlorine	flour
CARRIER OR EXTRACTION SOLVENTS	used to dissolve flavors, colors and spices	ethyl alcohol	vanilla extract
COLORS (natural and synthetic)	give food an appetizing appearance	carotene	butter, cheese
FOAMING OR WHIPPING AGENTS	enable pressure-packed products to be dispensed in a whipped state	nitrogen	dessert toppings
FIRMING AND CRISPING AGENTS	maintain the texture of fruits and vegetables	calcium chloride	canned vegetables
FOOD ENZYMES	act as catalysis to initiate desired chemical reactions	rennet	curdling milk in the making of cheese
HUMECTANTS	maintain desired moisture levels	sorbitol	shredded coconut, marshmallows
NON-NUTRITIVE SWEETENERS	provide a sweet taste, but contain no calories or nutrients	saccharine aspartame (?)	carbohydrate-reduced, sugar-free, calorie-reduced and low-calorie products
pH-ADJUSTING AGENTS (acids, alkalis and buffering agents)	control the acidity or alkalinity (pH) of foods; modify flavor slightly	sodium bicarbonate	baking powder
PRESERVATIVES -antimicrobal agents -antioxidants	inhibit the growth of molds, yeasts or bacteria prevent rancidity and oxidative discoloration	sodium diacetate butylated hydroxytoluene (BHT)	bread cooking oils
RELEASE AGENTS	help food separate from surfaces it touches during manufacturing or transport	mineral oil	baked goods (to remove from baking pans without sticking or crumbling)
TEXTURE MODIFYING AGENTS (emulsifiers, gelling agents, stabilizers and thickeners)	impact and maintain a desired consistency in foods	mono and diglycerides	ice cream

enzyme inhibitors present in plants which are protein in nature. The protease inhibitors have the ability to inhibit the proteolytic action of certain enzymes and are the most common in plants, especially in the legumes. Soya beans have been studied extensively because of their role in animal and human nutrition and are reported to inhibit bovine, porcine, ovine and human trypsin. Presence of trypsin inhibitors in raw soya bean have been shown to cause hypertrophy of the pancreas and increased losses of endogenous essential amino acids. Fortunately most of the plant protease inhibitors are destroyed quite readily by heat. Restoration of the nutritional properties of heated soya bean meals is attributed to the destruction of these inhibitors. In recent years a great deal of interest has centered around the use of plant protein isolates in the formulation of texturized foods. Work with soya bean isolates has shown that enzyme inhibitors are not completely removed in the process of extraction, precipitation and isolation of the proteins. Further heat treatment was necessary to improve their nutritive value. However, many inhibitors were also shown to have activity against chymotrypsin. These inhibitors are normally present in the human colon. The nutritional significance of these endogenous inhibitors is not fully understood.

In addition to protease inhibitors, plants also contain other enzyme inhibitors such as amylase inhibitors. If not properly inactivated, they can effectively impair the digestion and utilization of complex carbohydrates in the diet /51/. The most extensively studied naturally occurring non-protein enzyme inhibitors are the cholinesterase inhibitors. They are active against acetylcholine-esterases. Examples of such inhibitors are the glycoalkaloids of potatoes, α-solanine and α-chaconine. The clinical symptoms of toxicity in humans and animals include gastrointestinal disturbances and certain neurological disorders.

10.2 Phytohemagglutinins (lectins)

These are glycoproteins which are capable of agglutinating red blood cells. It is postulated that they may combine with the cells lining the intestinal walls causing nonspecific interference with nutrient absorption.

10.3 Saponins

These are glycosides occurring in a wide range of plants including spinach, beetroot, asparagus and alfalfa. They tend to lower the surface

tension of body fluids and may cause hemolysis, hence are considered to be highly toxic. The hydrolysis of saponins destroys the potential toxicity and yields sapogenins and sugar. It has been shown that dietary alfalfa saponins form complexes with cholesterol and thus help to lower the plasma cholesterol levels in chicks and monkeys. Suggestions have also been made that certain saponins may play an important role in the treatment of hypercholesterolemia /56/.

10.4 Glucosinolates

These are commonly known as goitrogens and are distributed throughout the plant kingdom, especially in the Cruciferae family (cabbage, turnip, rutabaga, mustard greens and rapeseed) /51/. They may be a causative factor in hypothyroidism with enlargement of the thyroid gland. Pro-goitrins in the plants are hydrolyzed by the thio-glucosidase enzyme system to produce an organic aglucone, glucose and bisulfate. The thyroid-inhibiting effect of the active ingredient, goitrin (5-vinyloxazolidine-2-thione) is thought to be due to the inhibition of organic binding of iodine. The unstable organic aglucone may undergo an intermolecular rearrangement to give an active isothiocyanate. Thioglucosidases can be inactivated by steaming the plants.

10.5 Cyanogens and cyanogenetic glycosides

Cyanide in trace amounts is widespread in the plant kingdom and occurs in the form of cyanogenic glucosides. Cyanide, a potent respiratory inhibitor, is released upon treatment with acids or appropriate hydrolytic enzymes. Relatively high concentrations are found in certain grasses, pulses, root crops and fruit kernels. Although most of these are consumed by animals, some do play an important role in human nutrition and health. The site of inhibition is the enzyme cytochrome oxidase, the terminal respiratory catalyst of aerobic organisms. Three distinct glucosides have been identified in edible species of plants, amygdalin, dhurrin and linamarine. Amygdalin is present in the kernels of stone fruits, such as bitter almonds, peaches and apricots. Several documented cases of cyanide poisoning by lima beans and casava are also known /36/.

10.6 Lathyrogens

These are found in the seeds of *Lathyrus* species of plants, e.g. chick peas. These seeds are habitually consumed in Asia, Southern Europe and the Middle East. Large consumption of *Lathyrus sativus* seed for a prolonged period causes lathyrism, a neurological disorder exhibiting muscular rigidity, weakness and paralysis; in severe cases death may ensue /36/. The toxins can be removed from the seeds by soaking them overnight in cold water or by steaming.

10.7 Gossypol

The polyphenolic gossypol pigment is present largely in cotton seeds at a level of 2.4-4.8% of the kernel. Characteristic symptoms of gossypol toxicity include loss of appetite, weight loss, hypoprothrombinemia, diarrhea and hair discoloration. It may also lower hemoglobin levels, decrease red cell count, serum protein, produce edema in lungs and heart, and degenerative changes in liver and spleen, small intestine and stomach. In addition, during the processing of the cotton seed, gossypol interacts with protein, thereby reducing the biological availability of lysine and the overall quality of the protein.

10.8 Favism

This condition is characterized by acute hemolytic anemia in susceptible individuals following ingestion of broad beans (fava beans) or inhalation of pollen of the *Vicia faba* plant. The causative agents have been identified as divicine and isouramil which are the aglycones obtained from the respective glycosides vicine and convicine by mild acid hydrolysis or by enzymatic splitting by α-glucosidase. Favism is caused by an inborn error in metabolism, especially in individuals lacking glucose-6-phosphate dehydrogenase (G6PDH). Fava beans are grown and consumed all over the world as a cheap and popular staple food. They contain 25% protein per edible portion of mature dry seeds. Research efforts are being focused on understanding the nature of the toxic agents in fava beans.

11. TOXICANTS PRODUCED BY FOOD SPOILAGE

Most food products consumed are highly perishable and as such may be easily spoiled and so deteriorate. Some sorts of deterioration

are accompanied by the production of toxic agents, while others inflict losses in the nutritive value of the food. The principal causes of spoilage in foods include growth of micro-organisms, the action of naturally-occurring enzymes in the food, chemical reactions, physical degradation and desiccation. Other factors that regulate the type and extent of microbial food spoilage are the pH, water content, temperature, oxygen concentration, degree of contamination with spoilage organisms and the presence of growth inhibitors in the food /21/.

The growth of certain micro-organisms has been identified as the major hazard associated with our food supply. It is difficult to assess the true extent of microbiological hazard, but it is generally believed that at least one out of every ten people suffers from the adverse effects of microbe-contaminated foods each year. Two types of microbiological hazards associated with our foods are recognized, namely, food infection and food intoxication.

11.1 Food infection

This is caused by the consumption of food containing large numbers of harmful microorganisms, mostly bacteria. Once ingested, the bacteria infect the intestinal tract. The illness is characterized by a violent form of stomach upset, diarrhea and frequent vomiting. The time between consumption of the food and the appearance of symptoms varies with the type of bacteria. Some important examples of bacteria causing food-borne infection include *Clostridium perfringens, Salmonella, Shigella, Escherichia coli, Streptococcus* and *Vibrio parahaemolyticus.* In addition to bacteria, several parasites and phages are also responsible for food-borne infection.

11.2 Food intoxication

Staphyloccccus aureus and *Clostridium botulinum* are widely known culprits which cause food intoxication if ingested. These bacteria have the ability to grow and produce toxins in food. Most of the bacteria are easily killed by heat during the processing and cooking of the food. However, some of the toxins produced by the bacteria are heat stable. Mycotoxins have been implicated in food by-products. In humans, toxicosis may be caused by the presence of molds including *Aspergillus*, *Penicillium*, *Fusarium* and others. Food intoxication has been reported due to *Aspergillus flavus* contamination of animal feed and aflatoxin elaborated by the mold. Major aflatoxins produced by

Aspergillus flavus include aflatoxins B_1, B_2, G_1, G_2. Furthermore, other aflatoxins, M_1, M_2, B_{2a}, G_{2a}, R and B_3, have also been implicated in various incidences of food intoxication. Aflatoxin B_1 appears to be the most potent of the aflatoxin hepatocarcinogens. Moderate to high incidence of hepatomas and hepatocellular carcinomas have occurred in rats receiving only about 15 ppb aflatoxin B_1 in the diet continuously. There is sufficient evidence now available suggesting aflatoxins as a major cause of adverse health effects in humans. Numerous reports from various countries indicate a definitive role of aflatoxin in human carcinoma /38/. The reactive metabolites of aflatoxin B_1 covalently bind to the cellular macromolecules, RNA, DNA and proteins, resulting in mutations, carcinomas and ultimately death.

Other important mycotoxins include patulin, citrinin, penicillic acid, luteoskyrin, islanditoxin, trichothenes, aspergillic acid, cyclopiazonic acid, zeralenone and sterigmatocystins. Numerous other toxic metabolites are also elaborated in food by various other molds.

12. TOXIC AGENTS PRODUCED BY FOOD PROCESSING OPERATIONS

Food processing operations should improve the quality of food and ensure safety for consumers. However, some processes may modify the chemical nature of the food which may cause harmful effects when consumed. The contaminants formed during processing may lower the quality of food products stored over a long period of time. FDA regulations now require manufacturers to list ingredients, nutritional values, and shelf-life on the labels of food products.

Pasteurization, boiling, sterilization, evaporation, fermentation, irradiation and the use of bleaching agents, acids, alkalis and the various types of solvents employed in commercial food processing, may affect the nutritive values of food products. The most widely studied are thermally-oxidized fats. Chemical changes in fats and oils subjected to high temperatures in the presence of oxygen cause the formation of a number of hydroperoxides, aldehydes, ketones, alcohols, epoxy acids, dihydroxy acids, keto acids, lactones, cyclic monomers and polymers /66/. The effects of both primary and secondary oxidation products are toxic when consumed at high concentrations; the fraction consisting of branched-chain fatty acids and cyclic compounds has been shown to have adverse health effects. The polymeric compounds once formed induce losses in the nutritive value of fats and

oils either due to poor absorption or by complexing with other nutrients, such as proteins, in the food. Thermally-oxidized fats have been shown to contain weakly carcinogenic compounds or compounds with strong co-carcinogenic properties. It is generally accepted that industrial processing operations such as refining, bleaching, deodorization, hydrogenation, frying, baking and roasting, which do not cause severe chemical changes in products, give no problems when these are ingested at low concentrations. Another type of chemical interaction in food, or nonenzymatic browning reactions, namely 'Maillard reactions', may be induced by high temperatures /44/.

13. EXPERIMENTAL ANIMAL DIETS

Diets for experimental animal studies are especially important in long-term toxicity/carcinogenicity studies of drugs and chemicals, where a low background incidence of spontaneous tumorigenicity and other diseases states is desirable. The different types of standard diets available are: (1) natural ingredient formulae (oats, soya, fishmeal); (2) semi-purified diets (casein, starch, corn oil, vitamin and mineral mixes), and (3) synthetic diets (specific amino acids, sugars, starches, lipids, etc.). However, the purity and stability of the dietary components are critical and there have been many instances where lack of care in the formulation, preparation or sterilization of the diet have had disastrous consequences in long-term studies, e.g. nitrosamine contamination of fishmeal, autoxidized lipids, depletion of vitamins during sterilization by irradiation, etc. The results of carcinogenicity studies of new drugs have been known to be completely reversed by changes in diet, e.g. by changing the level of dietary riboflavin in the carcinogenicity testing of a new azo pharmaceutical (riboflavin enhances microbial azo reductase), or feeding diets with low nitrate and high ascorbate contents in the testing of new gastric acid inhibitors (low stomach pH can result in microbial overgrowth which reduces nitrate to nitrite, and can thus nitrosate dietary amines to carcinogenic nitrosamines, a process inhibited by ascorbate). The amount and nature of dietary fiber may have marked effects on the observed rodent toxicity of many drugs and chemicals /65,82/.

A complete purified diet (AIN-76) was developed for rodent studies, but this diet was found occasionally to be haemorrhagic, due to the destruction or inhibited utilization of its vitamin K (menadione) content, to result in nephrocalcinosis due to mineral imbalance, and the

high sucrose content of the diet resulted in dental caries. A modified diet (MODAIN) was developed which contains maize instead of sucrose, a ten-fold increase in vitamin K, and a four-fold increase in the magnesium content to reduce the incidence of nephrocalcinosis /82/.

The addition of brewers yeast to laboratory animal chows leads to the incorporation of certain hop components (lupulones) which result in the induction of cytochrome P4503A and a decrease in chemically-induced tumors /57/, thus confusing the findings in rodent carcinogenicity studies.

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