V Sources and Biogenesis of Trace Organic Constituents of the Diet

By S. A. Slorach
TOXICOLOGY LABORATORY, NATIONAL FOOD ADMINISTRATION, BOX 622, S-751 26 UPPSALA, SWEDEN

1 Introduction

Thousands of organic compounds having widely differing chemical structures and originating from a variety of sources are present in trace amounts in our diet. A few of these substances are essential to health, whilst others can produce toxic effects in man even at low levels of intake. However, most of them are neither essential nor apparently hazardous to health in the amounts normally ingested. In this paper the compounds have been grouped according to their sources, proceeding from the farm or fishing ground to the fork. However, it must be borne in mind that some substances may enter food by several different pathways. The number of organic compounds present in trace amounts in the diet is so large that it is possible to give only a few examples from each group here, and the emphasis has been placed on substances of toxicological interest.

2 Substances Normally Synthesized by Plants and Animals

A. Vitamins and Anti-vitamin Factors.—Plants are able to synthesize certain vitamins which are essential to human health (*e.g.* vitamins B_1, B_2, B_6, C, E, and K), whilst others (*e.g.* retinol, cholecalciferol, and vitamin B_{12}) are synthesized by animals from precursors in their diet and are present in foods of animal origin such as liver and milk. Raw soya beans and kidney beans contain anti-vitamin factors, the chemical nature of which has not yet been fully elucidated.

B. Protease Inhibitors and Lectins.—Raw soya beans and some other foods derived from leguminous plants contain protease inhibitors and haemagglutinins (lectins) which are toxic to man. Fortunately they are thermolabile proteins and thus destroyed by cooking. Broad beans contain toxic principles which can cause acute haemolytic anaemia in persons deficient in the enzyme glucose-6-phosphate dehydrogenase. The nature of the toxic principle(s) has not yet been definitely established, but the closely related glycosides vicine [2,6-diamino-4,5-dihydroxy-pyrimidine 5-(β-D-glucopyranoside)] and convicine seem likely candidates.[1]

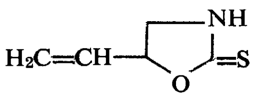
C. Lathyrogens.—The seeds of certain *Lathyrus* species, for example *L. sativus*

[1] J. Mager, M. Chevion, and G. Glaser in 'Toxic Constituents of Plant Foodstuffs', 2nd edition, ed. I. E. Liener, Academic Press, New York, 1980.

(chick-pea), contain a neurotoxin (β-*N*-oxalyl-L-α,β-diaminopropionic acid) which is probably the chief factor responsible for causing neurolathyrism.[2] Outbreaks of this disease, which is characterized by spastic paralysis of the legs as a result of damage to the spinal cord, have occurred in countries where chick-peas and other lathyrogen-containing foods are habitually consumed.

D. Cyanogenetic Glucosides.—The cyanogenetic glucosides amygdalin, dhurrin, linamarin, and lotaustralin are present in plant foodstuffs such as bitter almonds, sorghum, and cassava. Complete hydrolysis of amygdalin yields glucose, benzaldehyde, and hydrogen cyanide, which give almond products their characteristic flavour. Enough cyanide can be produced from the amygdalin in ten bitter almonds to poison a child.

E. Glucosinolates (Thioglucosides).—Many cruciferous plants, such as rape, kale, cabbage, mustard, and horseradish, contain glucosinolates (thioglucosides). These compounds (or their hydrolysis products) are responsible for the pungent flavours of horseradish and mustard, and they may also be associated with endemic goitre, although this has not been conclusively demonstrated. The most important glucosinolate in rapeseed is 2-hydroxy-3-butenylglucosinolate (also called progoitrin). Enzymatic hydrolysis of this compound yields 5-vinyloxazolidine-2-thione (goitrin) (1), nitriles, glucose, and sulphate. If rapeseed is included in cattle feed, some of the progoitrin present will be converted to goitrin and secreted in the milk. However, it is unlikely that the levels found in milk (*e.g.* 20 $\mu g\ l^{-1}$ in a recent Swedish study[3]) imply a risk for goitre in man.



(1)

$$Me{-}N{=}N{-}CH_2OH \quad (N \downarrow O)$$

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F. Cycasin.—The glucoside cycasin has been isolated from cycad plants, which are used as a source of food starch in some countries. Cycasin is hydrolysed in the gut by bacterial β-glucosidase to yield the aglycone methylazoxymethanol (2), which has been found to be carcinogenic in animals.

G. Pyrrolizidine Alkaloids.—Coltsfoot and comfrey, which have been used as foods or herbal remedies, contain pyrrolizidine alkaloids, which produce liver cancer when fed to rodents.

H. Gossypol.—Cottonseed contains polyphenolic pigments called gossypol, the

[2] G. Padmanaban in 'Toxic Constituents of Plant Foodstuffs', 2nd edition, ed. I. E. Liener, Academic Press, New York, 1980.

[3] E. Josefsson, *Var Foeda*, 1979, **31**, 471.

presence of which in cottonseed oil or meal reduces their value in human and livestock nutrition owing to their toxicity. However, it is interesting to note that gossypol is presently being tested in China as a potential antifertility agent in men.

I. Solanine and Other 'Stress Metabolites' in Potatoes.—Specific stress (*e.g.* attack by disease organisms) or non-specific stress (*e.g.* mechanical injury or exposure to light) may have profound effects on the chemical composition of potatoes. The levels of 'stress metabolites' such as the glycoalkaloids α-solanine and α-chaconine, steroids, and sesquiterpenes are of especial interest.[4] As recently as 1978 a large outbreak of suspected solanine poisoning was reported in London following the consumption of potatoes stored in a school kitchen over the summer holidays.[5] A new variety of potato, Lenape, which was ready for introduction in the U.S.A. about eight years ago, was found to cause illness when ingested. It was found to produce much higher levels of glycoalkaloids than did other commercial varieties and fortunately was withdrawn before it came to widespread use.

J. Flavourings.—It may come as a surprise to some 'health food' addicts that the flavour of even 'organically grown' fresh fruits and vegetables is due to the presence of complex mixtures of small amounts of hydrocarbons, alcohols, aldehydes, ketones, acids, esters, and other organic compounds. For example, citrus fruits contain over 200 different volatile organic constituents synthesized by the plants themselves. Thousands of natural, nature-identical, and artificial flavouring substances are now used to flavour food (see ref. 6). Some natural flavours contain toxic substances, such as safrole, β-azarone, and coumarin, and maximum permitted levels for these compounds in foodstuffs have been laid down in some countries.

K. Colours.—The natural colours of foods are due to the presence of small amounts of organic compounds, such as carotenoids (yellow, orange, red), anthocyanins (red-blue), betanine (red-blue), and chlorophyll (green). The increasingly restrictive attitude of the food-control authorities in some countries towards the use of azo-colours has led to an increasing interest from the food industry in the use of colours derived from natural sources or their synthetic equivalents.

3 Environmental Pollutants

Air, soil, and water pollution may result in the contamination of food plants and animals with organic compounds that are toxic to man or that affect the organoleptic properties of the food, or both.

[4] S. F. Osman, R. M. Zacharius, E. B. Kalan, T. J. Fitzpatrick, and S. Krulick, *J. Food Protect.*, 1979, **42**, 502.
[5] M. McMillan and J. C. Thompson, *Q. J. Med.*, 1979, **48**, 227.
[6] *Chem. Soc. Rev.*, 1978, **7**, 167.

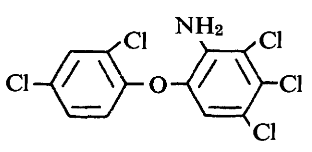
A. Polycyclic Aromatic Hydrocarbons.—The combustion of fossil fuels in power stations, motor-vehicle engines, *etc.*, and the use of carbon electrodes in the production of aluminium lead to atmospheric pollution with polycyclic aromatic hydrocarbons (PAHs). Food plants, especially leafy vegetables, grown in the vicinity of such sources of pollution often contain elevated levels of carcinogenic PAHs, such as benz(a)pyrene, benz(e)pyrene, and benzo(b)fluoranthene.

B. Organochlorine Compounds.—The use of stable organochlorine compounds, such as DDT, hexachlorobenzene, hexachlorocyclohexane isomers, and toxaphene, in agriculture and vector control has led to widespread environmental pollution with these substances. Because of their lipid solubility and stability they accumulate as they pass up food chains. They are found as contaminants in many foodstuffs of animal origin, such as fish, milk, and butter. DDE, the main metabolite of DDT, is held very tenaciously in adipose tissues, and the level of DDE in foods of animal origin is usually higher than that of the parent compound.

Polychlorinated biphenyls (PCBs), which have a variety of industrial uses, have long been recognized as global pollutants of the aquatic environment. Although PCBs are very stable compounds, during their passage through food chains there is a progressive loss of lower chlorinated compounds due to selective biotransformation. For this reason the PCB pattern in, for example, fish is not the same as that in the commercial PCB mixture which has been discharged into the environment. In most industrialized countries PCBs are present in fish, butter, and other foods of animal origin. Breast-fed children are exposed to PCBs *via* their mothers' milk.

In the sixties fish in the River Viskan downstream of the textile factories in Borås in Sweden were found to contain high levels of dieldrin, which was used as a mothproofing agent. The authorities then prohibited the use of dieldrin for this purpose and the levels in fish dropped. However, subsequently two other pollutants, previously unobserved, appeared on the chromatograms in the fish analysis. These were identified as 2′,3,4,4′,5-pentachloro-2-aminodiphenyl ether (3) and its 2′,3,4,4′,5,6-hexachloro analogue, both of which are closely related to the active ingredients of one of the mothproofing agents which replaced dieldrin.[7] Thus the contamination problem was not solved, just changed.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most toxic substances



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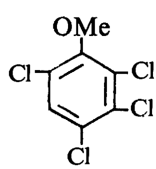

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[7] G. Westöö and K. Norén, *Ambio*, 1977, **6**, 232.

known, may be present as an impurity in the herbicide 2,4,5-trichlorophenoxyacetic acid. Chlorinated dioxins and dibenzofurans can also be formed by pyrolysis or burning of chlorophenols and PCBs. The accidental discharge of TCDD from a factory in Seveso in Italy led to TCDD contamination of cows' milk in surrounding areas with this substance.[8] Tetra- and penta-chlorophenols are used as wood preservatives, and the use of shavings of preserved wood as litter in broiler houses can lead to the production of off-flavour or taint in poultry meat. This has been traced to the presence of chloroanisoles, *e.g.* 2,3,4,6-tetrachloroanisole (4), produced from the chlorophenols by microbial action.[9]

C. Methylmercury and Organoarsenicals.—Pollution of the aqueous environment with mercury discharged from, for example, chlorine–alkali factories where mercury electrodes are used, results in increased levels of methylmercury in fish. Inorganic mercury is converted to methylmercury by micro-organisms in sediments; it passes up the food chain and can reach high levels in carnivorous fish, such as pike, tuna, and sharks. Because of its lipid solubility it is readily absorbed from the human gastro-intestinal tract, enters the brain, and crosses the placenta to the foetus. The epidemics of poisoning in Minamata and Niigata in Japan caused by the ingestion of fish contaminated with methylmercury provide a grim warning of the necessity of monitoring fish for this compound.

Marine pollution with arsenic leads to elevated levels of highly stable organoarsenicals in fish (especially flatfish) and crustaceans. It must also be borne in mind that we know very little about the chemical form in which many other metals are present in food plants and animals. It is quite possible that some are present as organometallic compounds or complex-bound to organic compounds.

D. Other Pollutants.—The accidental or intentional discharge of crude oil into the marine environment results in the contamination of fish and shellfish with hydrocarbons and other constituents of crude oil.

Certain planktonic unicellular organisms, known as dinoflagellates, produce a toxin which can be taken up by filter-feeding bivalve molluscs, such as mussels and clams. Consumption of contaminated mussels can result in paralytic shellfish poisoning in man. The major toxins responsible for this appear to be saxitoxin and its 11-hydroxy derivative.[10]

4 Residues of Veterinary Drugs and Feed Additives

A wide range of drugs are administered to food-producing animals to treat or prevent diseases or to improve feed conversion, *etc.* Unless adequate holding (quarantine) times are observed between administration of the drug and taking the milk, eggs, carcass, *etc.*, for food use, trace amounts of the drug or its

[8] R. Fanelli *et al.*, *Bull. Environ. Contam. Toxicol.*, 1980, **24**, 634.
[9] U. G. Ahlborg and T. M. Thunberg, *Crit. Rev. Toxicol.*, 1980, **7**, 1.
[10] Report of the W.H.O. Expert Consultation on Paralytic Shellfish Poisoning, West Berlin, 5—8 December 1978, World Health Organization, Geneva, 1979.

metabolites may be present in the food when it reaches the consumer. Examples of the antimicrobial agents used are penicillins, streptomycin, tetracyclines, bacitracin, chloramphenicol, nitrovin, and sulphonamides. Dichlorvos and coumaphos are examples of organophosphorus anthelminthics in veterinary use. The use of hormones, both natural and synthetic (*e.g.* diethylstilboestrol), in food-producing animals such as calves and pigs is currently the subject of intense debate in Europe. Poultry feed usually contains coccidiostatics, *e.g.* amprolium, clopidol, or dinitolmide, to prevent outbreaks of coccidiosis. The possibility that poultry waste will find increasing use in feedingstuffs for other food-producing animals means that the levels of residues of feed additives in such waste are also of interest to the food-control authorities. Citranaxanthin, a synthetic carotenoid, is added to poultry feed in some countries to 'improve' the colour of the egg yolk and to colour the carcass.

5 Pesticide Residues

Virtually all the cereals, fruit, vegetables, and sugar beet grown commercially in the U.K. are treated with pesticides. Based on the quantity of active substance used, the most important group is the herbicides, followed by fungicides and insecticides. Although large quantities of pesticides are used in agriculture, only trace amounts are present in the food when it is consumed, if the pesticide has been used according to Good Agricultural Practice. In recent decades there has been a move away from the use of persistent organochlorine insecticides, such as DDT, dieldrin, hexachlorobenzene, and hexachlorocyclohexane isomers, although toxaphene is widely used in the U.S.A. As has already been mentioned, these compounds are widely distributed in the environment and give rise to food contamination problems in fish and other foods of animal origin. Organophosphorus insecticides, such as malathion, parathion, bromophos, and dichlorvos, break down more rapidly and present less of an environmental problem but are more dangerous to the user because of their higher acute toxicity.

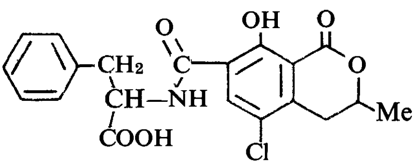
Pyrethrins from natural sources and synthetic pyrethroid derivatives are being used increasingly as insecticides. Amongst the most widely used herbicides are 2,4-dichlorophenoxyacetic acid (2,4-D) and its trichloro analogue (2,4,5-T). Bis(ethylene dithiocarbamates), such as maneb and zineb, are widely used as fungicides. Some of them can be degraded to ethylenethiourea, which has been shown to produce thyroid tumours in experimental animals. Because they are used post-harvest on, for example, citrus fruits the anti-fungal agents diphenyl, *o*-phenylphenol, thiabendazole, and benomyl can be present in trace amounts in treated food when it is ingested.

The treatment of spices to kill insects and micro-organisms presents special problems. One of the currently used methods – gassing with ethylene oxide – can leave residues of this mutagen or its reaction products, *e.g.* ethylene chlorohydrin, in the final product.

6 Bacterial Toxins and Mycotoxins

A. Substances Produced by Bacteria.—The growth of certain bacteria on foods can give rise to toxins, such as *Clostridium botulinum* toxin and staphylococcal α-toxin. These substances can cause illness or even death when ingested. If certain bacteria (*e.g. Proteus* organisms) are allowed to grow on the muscle tissue of scombroid fish (such as tuna and mackerel) when it is not held at a low enough temperature, the amines histamine, cadaverine, putrescine, and spermidine are formed. The ingestion of fish spoiled in this way can give rise to so-called 'scombroid poisoning', although it is still not certain which substance(s) is the causative agent.

B. Mycotoxins.—The growth of certain moulds on foodstuffs can lead to the production of toxic mould metabolites called mycotoxins. Aflatoxins, a group of closely related mycotoxins produced by the growth of *Aspergillus flavus*, are found in mouldy nuts and maize. Aflatoxin B_1 has been shown to be carcinogenic in animals, and there is epidemiological evidence that it can produce primary liver cancer in man. If cow's feed is contaminated with aflatoxin B_1, the milk contains low levels of its metabolite aflatoxin M_1. Aflatoxins are mainly a problem in foods grown in hot, humid climates, but other mycotoxins are also of current interest in Europe. Ochratoxin A (5), produced by the growth of the moulds *Penicillium viridicatum* and *Aspergillus ochraeus* on grain, has been found in the blood and kidneys of pigs fed mouldy grain, which has led to the rejection of pig carcasses at slaughterhouses. Patulin has been detected in mouldy apples and products thereof. Sterigmatocystin, zearalenone, and deoxynivalenol (vomitoxin) (6) are examples of other mycotoxins which can be produced by the growth of moulds on grain and which are of current concern to the food-control authorities.



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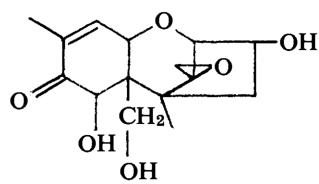

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7 Intentional Food Additives

Most intentional food additives are used in more than 'trace' amounts and will therefore not be discussed here. However, in some cases undesirable substances may be present in them in trace amounts as impurities. For example azo-colours, such as tartrazine, amaranth, and Brown FK, contain small amounts of subsidiary dyes and other organic impurities. One of the most widely used food colours, that unholy mixture known as caramel colour produced by the ammonia process, contains an as yet unidentified anti-pyridoxine factor, in addition to 4-methylimidazole and a range of other heterocyclic compounds.

8 Substances Introduced During Processing or Preparation

A. Substances Introduced by Smoking.—Meat and fish are exposed to smoke generated by the partial combustion of hardwoods to give them a characteristic flavour and colour and to extend their shelf life. Traditional smoking introduces into the smoked food trace amounts of a large number of organic compounds, including phenols, acids, aldehydes, ketones, and furans. Smoked food also contains trace amounts of carcinogenic polycyclic aromatic hydrocarbons, such as benz(a)pyrene. The chemistry of smoked foods and smoke flavours has been reviewed by, *inter alia*, Gilbert and Knowles.[11]

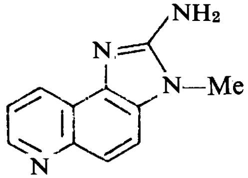
B. Nitrosamines in Nitrite-treated Foods.—Nitrosamines can be formed in foods or in the human body by reaction between nitrite and, for example, secondary amines. Many nitrosamines have been found to be carcinogenic in animals, and this has stimulated investigations into their presence in food.[12] Volatile nitrosamines have been studied most extensively since methods for their analysis were developed first. Fried bacon contains mainly *N*-nitrosopyrrolidine (1—10 $\mu g\ kg^{-1}$) and small amounts of *N*-nitrosodimethylamine (NMDA) and *N*-nitrosopiperidine.[13] Fish and cheese may also contain low levels of NMDA. In studies in West Germany and the U.K. beer has earlier been found to contain relatively high levels of NMDA, originating from malt dried by direct firing, a process in which nitrous gases produced during firing react with amines in the malt. However, this problem has now been largely overcome either by using indirect firing or by adding sulphur dioxide to the hot gases used for drying the malt.

C. Residues of Freezing Agents or Solvents.—When direct-contact freezing agents, such as dichlorodifluoromethane, are used to freeze foodstuffs, trace amounts of these compounds may be present in the food as sold and, unless it is adequately heated beforehand, also when it is consumed. Similarly, traces of solvents used in fat fractionation (*e.g.* 2-nitropropane) may be present in food.

D. Compounds Produced by Heating Meat, Fish, *etc*.—Recent work by Sugimura and his group in Tokyo has shown that pyrolysis of amino-acids, such as tryptophan and glutamic acid, or broiling fish or meat results in the production of potent mutagens.[14] There is some evidence that part of the mutagenic activity in the broiled fish is due to the presence of 2-amino-3-methylimidazo[4,5-f]quinoline (7) and a methyl derivative thereof.[15] Two other highly potent mutagens, namely 3-amino-1,4-dimethyl-5*H*-pyrido[4,3b]indole and its 1-methyl analogue (8), have been isolated from tryptophan pyrolysates and broiled sardines.[16] This is a very rapidly developing area of research and much has still to be learnt about the

[11] J. Gilbert and M. E. Knowles, *J. Food Technol.*, 1975, **10**, 245.
[12] L. Fishbein, *Sci. Total Environ.*, 1979, **13**, 157.
[13] K. S. Webb and T. A. Gough, *Oncology*, 1980, **37**, 195.
[14] T. Sugimura and M. Nagao, *Crit. Rev. Toxicol.*, 1979, **6**, 189.
[15] H. Kasai *et al.*, *Proc. Jpn. Acad.*, 1980, **56**, 278.
[16] Z. Yamaizumi *et al.*, *Cancer Letters*, 1980, **9**, 75.

identity, occurrence, and health significance of mutagens formed during the industrial processing and domestic preparation of food.



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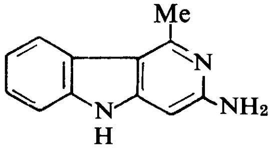

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9 Substances Migrating from Packaging Material

Plastics are now widely used as food packaging materials. Various constituents of such materials may migrate to food and thus affect its organoleptic properties or render it unfit for human consumption for toxicological reasons. In recent years interest has centred on the monomers vinyl chloride, styrene, vinylidene chloride, and acrylonitrile. Such monomers have been found, usually at levels below 10 μg kg^{-1}, in foods packed in plastics in which they are present as residual monomers.[17,18] Other substances of current interest are phthalates and adipates used as plasticizers in food-contact materials. The fact that certain phthalates and adipates have been shown to be carcinogenic in animals provides an extra incentive to study their occurrence in food.

10 Conclusions

Traces of organic compounds in our diet contribute to its nutritive value and to a large extent govern its organoleptic properties. However, some trace organic constituents are potentially harmful and must be subject to control in order to ensure the safety of food. Much more research, in both chemistry and toxicology, is necessary to provide a firmer foundation for the evaluation of the health risks posed by recently discovered food constituents, for example those produced during frying and grilling meat and fish.

[17] The Third Report of the Steering Group on Food Surveillance, The Working Party on Vinylidene Chloride, Food Surveillance Paper No. 3, H.M.S.O., London, 1980.

[18] The Second Report of the Steering Group on Food Surveillance, The Working Party on Vinyl Chloride, Food Surveillance Paper No. 2, H.M.S.O., London, 1978.