Review
The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods
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Abstract: The glucosinolates are a large group of sulphur-containing compounds which occur in all the economically important varieties of Brassica vegetable. Their common structure comprises a β-D-thioglucose group, a sulphonated oxime moiety and a variable side-chain derived from methionine, tryptophan or phenylalanine. When the plant tissue is damaged the glucosinolates are hydrolysed by the endogenous enzyme ‘myrosinase’ (thioglucoside glycohydrolase EC 3:2:3:1), to release a range of breakdown products including the bitter, biologically active isothiocyanates. Although these compounds exert antinutritional effects in animals there is also substantial evidence that they are the principal source of anticarcinogenic activity in Brassica vegetables, and this provides a strong motive for the manipulation of glucosinolate levels in vegetables for human consumption. This review provides an overview of the evidence for a beneficial role for glucosinolates in human health, and describes the current state of knowledge regarding the genetics and biosynthesis of glucosinolates, their chemical analysis, their behaviour during cooking and processing, and their bioavailability to humans. As the genetic basis of glucosinolate biosynthesis becomes more apparent, and tools for marker-assisted plant breeding become more available, the selective breeding of horticultural brassicas with different levels and types of glucosinolates, whether by conventional means or genetic manipulation, is becoming a practical possibility. However before this strategy becomes commercially viable, the health benefits of glucosinolates for human beings must be unequivocally established.

INTRODUCTION
The discovery that the habitual consumption of a diet deficient in fruits and vegetables effectively doubles an individual’s risk of developing many types of cancer has focused attention on the causal mechanisms underlying this relationship.1,2 Fruits and vegetables are rich sources of micronutrients and dietary fibre, but they also contain an immense variety of biologically active secondary metabolites which provide the plant with colour, flavour and sometimes antinutritional or toxic properties.3,4 Amongst the most important classes of such substances are the carotenoids, flavonoids and more complex phenolics, saponins, phytosterols, glycoalkaloids and the glucosinolates. This review is concerned with the last of these, a large group of sulphur-containing compounds found in plant families of the order Cappar-ales, including the Brassicas, which include a large number of economically important vegetable varieties.

All the glucosinolates possess a common fundamental structure comprising a β-D-thioglucose group, a sulphonated oxime moiety and a variable side-chain derived from methionine, tryptophan, phenylalanine and some branched-chain amino acids.5 The glucosinolates are interesting chiefly because when the plant tissue is damaged by food preparation or chewing they are brought into contact with, and hydrolysed by, the endogenous enzyme ‘myrosinase’, (thioglucoside glycohydrolase EC 3:2:3:1), releasing a complex variety of breakdown products including isothiocyanates. These hot and bitter compounds, commonly termed ‘mustard oils’, are often volatile with an acrid smell.

The various effects of glucosinolates on the quality of both human and animal foods have encouraged
interest in their natural biosynthetic pathways, and in the possibility of manipulating glucosinolate levels to produce new and improved commercial varieties. The emerging evidence that brassica vegetables may have important anticarcinogenic effects associated with the biological activity of glucosinolate breakdown products provides another strong motive for the manipulation of the glucosinolate content of brassica vegetables for human consumption. In this review we present a brief overview of the evidence for a beneficial role for glucosinolates in human health and summarise the current state of knowledge regarding the biosynthesis of glucosinolates, their chemical analysis and behaviour during cooking and processing, and their bioavailability to humans.

CHEMICAL AND PHYSICAL PROPERTIES

The common structure of the glucosinolates is illustrated in Fig. 1. The structural diversity of the group is a consequence of the very considerable variety of substituents in the side-chain which occur in nature. Although over 100 different glucosinolates have been identified, they fall into three principal groups comprising the aliphatic group having an alkyl or alkenyl side-chain (sinigrin, progoitrin), the aromatic group (glucotusturtin) and the indolyl group (gluco-brassicin, neoglucobrassicin). The chemical structure and total content of glucosinolates vary between species, and between varieties within a species. Within any particular variety of vegetable, the glucosinolate content is influenced greatly by growing conditions. Despite the diversity of glucosinolate side-chains (Fig 1), only seven of these structures correspond directly to a protein amino acid (alanine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan). The remaining glucosinolates have side-chain structures which arise in three ways. Firstly, many glucosinolates are derived from chain-elongated forms of protein amino acids, notably from methionine and phenylalanine. Secondly, the structure of the side-chain may be modified after amino acid elongation and glucosinolate biosynthesis by, for example, the oxidation of the methionine sulphur to sulphinyl and sulphonyl, and by the subsequent loss of the o-methylsulphinyl group to produce a terminal double bond. Subsequent modifications may also involved hydroxylation and methoxylation of the side chain. Thirdly, some glucosinolates occur which contain relatively complex side-chains such as o-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate in Reseda odorata and glucosinolates containing a sinapoyl moiety in Raphanus sativus.

Glucosinolates remain chemically stable within the cytoplasm until brought into contact with myrosinase following tissue disruption. Upon hydrolysis, glucosinolates yield equimolar quantities of glucose, aglycone and sulphate, but the instability of the aglycones leads to further reactions, the main products of which are thiocyanates, nitriles and isothiocyanates. The pattern of reaction products varies with the structure of the side-chain and the reaction condition.

BIOLOGICAL FUNCTIONS IN PLANTS AND OTHER ORGANISMS

Glucosinolate breakdown products modify the behavioural and nutritional responses of both invertebrates, and vertebrate herbivores, to cruciferous plants. It is probably valid to regard glucosinolates as natural pesticides but the relationship between plant and herbivore often turns out to be very complex. High levels of glucosinolates have been shown experimentally to impose metabolic stress on generalist invertebrate herbivores, and to cause impaired growth. In the case of oilseed rape (Brassica napus ssp oleifera), a high glucosinolate content is associated with reduced grazing by slugs and pigeons, and there is evidence that in certain habitats the exposure of wild populations of Brassica oleracea to generalist herbivores imposes selection pressure for increased levels of aliphatic glucosinolates. However other invertebrate herbivores which specialise in Brassicas have become adapted to the presence of glucosinolates and are attracted to feed selectively on varieties containing specific compounds.

Human beings are also sensitive to the strong flavours of glucosinolate breakdown products, and these compounds are therefore important determinants of flavour in a variety of commercially important Brassicas. Allyl isothiocyanate is largely responsible for the characteristic hot flavours of condiments made from mustard and horseradish, and the glucosinolates sinigrin and progoitrin confer bitterness on Brussels sprouts and other brassica vegetables. One consequence of this is that glucosinolates are important target compounds for plant breeders who wish to modify the flavour of commercial varieties to satisfy particular consumer preferences.

Glucosinolate breakdown products exert a variety of toxic and antinutritional effects in higher animals, amongst which the adverse effects on thyroid metabolism are the most thoroughly studied. Both thiocyanate ions, and oxazolidine-2-thiones formed from glucosinolates with an aliphatic side-chain containing a beta-hydroxy group, are goitrogenic, although the mechanisms of action are different. Thiocyanate ions are iodine competitors, and therefore in both pigs and rats their goitrogenic effects can be mitigated to some extent by increasing the level of iodine in the diet. On the other hand 5-vinylazolinedione-2-thione is thought to be a direct inhibitor of thyroxine synthesis, an effect which is independent of iodine availability. The goitrogenicity of these compounds to domestic livestock is an important limiting factor in the commercial exploitation of brassica feedstuffs, and of rape seed in particular, and much research has been devoted to this issue. In principle, glucosinolate breakdown products are probably also capable of inducing
goitrogenic effects in humans, but there is little or no epidemiological evidence that this is an important cause of human disease. Experimentally, inclusion of 150 g of Brussels sprouts in the diets of adult volunteers had no effect on their levels of thyroid hormones, presumably because cooking had inactivated myrosinase and hence reduced the biological availability of the goitrogenic breakdown products to sub-clinical levels. There is a need for further studies of this type because the growing interest in the

<table>
<thead>
<tr>
<th>Amino acid precursor</th>
<th>Glucosinolate structure</th>
<th>Amino acid chain elongation</th>
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<tbody>
<tr>
<td>Methionine</td>
<td>Methylthioalkyl ( \text{CH}_3-S-\text{CH}_2-\text{CH}_2-[\text{CH}_2]_n-\text{GSL} )</td>
<td>( n = 1 - 8 )</td>
</tr>
<tr>
<td></td>
<td>Methylsulphinylalkyl ( \text{CH}_3-SO-\text{CH}_2-\text{CH}_2-[\text{CH}_2]_n-\text{GSL} )</td>
<td>( n = 1 - 9 )</td>
</tr>
<tr>
<td></td>
<td>Methylsulphonylalkyl ( \text{CH}_3-SO_2-\text{CH}_2-\text{CH}_2-[\text{CH}_2]_n-\text{GSL} )</td>
<td>( n = 1, 2, 6 )</td>
</tr>
<tr>
<td></td>
<td>4-Methylsulphinyl-3-butenyl ( \text{CH}_3-SO-\text{CH}=\text{CH}-[\text{CH}_2]_2-\text{GSL} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alkenyl ( \text{CH}_2=\text{CH}-[\text{CH}_2]_n-\text{GSL} )</td>
<td>( n = 1, 2, 3 )</td>
</tr>
</tbody>
</table>
|                      | 2-Hydroxy-3-butenyl \( \text{CH}_2=\text{CH}-\text{CH}_2-\text{GSL} \) | | \( \text{OH} \)
|                      | 2-Hydroxy-4-pentenyl \( \text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2-\text{GSL} \) | | \( \text{OH} \)
|                      | Hydroxalkyl \( \text{CH}_2-\text{CH}_2-[\text{CH}_2]_n-\text{GSL} \) | \( n = 1, 2, 3 \) |
|                      | Phenol \( \text{CH}_2-\text{CH}=\text{CH}-[\text{CH}_2]_n-\text{GSL} \) | \( n = 1 - 4 \) |
|                      | Benzoyloxyalkyl \( \text{CH}_2=\text{CH}_2-\text{GSL} \) | |
| Valine                | \( \text{CH}_3-\text{CH}-[\text{CH}_2]_n-\text{GSL} \) | \( n = 0 - 2 \) |
| Isoleucine            | \( \text{CH}_3-\text{CH}_2-\text{CH}-[\text{CH}_2]_n-\text{GSL} \) | \( n = 0, 1 \) |
| Phenylalanine         | Phenylalkyl \( \text{CH}_2-\text{CH}_2-[\text{CH}_2]_n-\text{GSL} \) | \( n = 0 - 4 \) |

Figure 1. (a) Examples of glucosinolates derived from elongated amino acids. \( n \) denotes the number of additional methylene groups (b) Examples of glucosinolates derived from non-chain-elongated amino acids.
anticarcinogenic properties of glucosinolate breakdown products discussed below, and the possibility that this may lead to increased human exposure to these compounds, raises important questions about the balance of adverse and beneficial effects of Brassica vegetables in the human diet.

Apart from their goitrogenic properties, glucosinolate breakdown products induce other less specific toxic effects in laboratory rodents, including embryotoxicity and impairment of growth, and enlargement of the liver without detectable evidence of histological changes. Certain glucosinolate breakdown products have also been under suspicion in the past as possible human carcinogens. Indole compounds such as indole-3-carbinol and indole-3-acetonitrile are formed from the hydrolysis of indoleglucosinolates in vegetables such as Chinese cabbage, and these can undergo nitrosation in the presence of nitrite to form mutagenic N-nitroso compounds. However the products do not appear to be sufficiently stable to cause a significant risk to human health under conditions relevant to the preparation and ingestion of food. Uncooked juices obtained from a variety of Brassica vegetables also induce mutations in microbiological assay systems and clastogenic changes in mammalian cell lines, induced directly by glucosinolate breakdown products including allyl and phenethyl isothiocyanate. However there is no evidence that these biomarkers are associated with any carcinogenic risk in intact animals or human beings in vivo. On the contrary, the epidemiological evidence strongly suggests that consumption of brassica vegetables is associated with reduced risk of cancer at many sites, including the alimentary tract, which seems likely to contain tissues which are the most heavily exposed of all to glucosinolate breakdown products.

In the largest and most detailed review of diet and cancer yet published, The World Cancer Research Fund concluded that diets rich in cruciferous vegetables probably protect human beings specifically against cancers of the colon, rectum and thyroid and, when consumed as part of diet high in other types of vegetable, generally against cancer at other sites. This epidemiological evidence is consistent with a host of experimental studies, which from the 1960s onwards...
have indicated that glucosinolate breakdown products exert anticarcinogenic activity in experimental animal models (See references 30, 31 for more detailed reviews).

Wattenberg 32 established that benzyl isothiocyanate, a breakdown product of the glucosinolate glucotropaeolin, would inhibit the induction of mammary tumours by 7,12-dimethylbenz(a)anthracene (DMBA) when administered orally to rats two hours before treatment with the carcinogen. This effect appears to be due to inhibition of carcinogen activation, so that the initial stage of the carcinogenic sequence associated with DNA damage is blocked,32,33 and hence substances which protect against experimentally induced tumours when given before or in conjunction with a chemical carcinogen are termed blocking agents.3,34 Conversely, substances which can inhibit the process of carcinogenesis when given some time after administration and full metabolism of the carcinogen are said to be suppressing agents.3,35 Wattenberg35 showed that purified benzyl isothiocyanate exhibited suppressing activity against DMBA-induced mammary tumours and later established that whole plant tissue of broccoli and cabbage, fed to rats starting one week after treatment with the carcinogen, would also suppress induction of mammary tumours.36 The most important blocking mechanism induced by glucosinolate breakdown products is probably modulation of the activities of Phase I and Phase II biotransformation enzymes which together catalyse a variety of hydrolytic, oxidation or reduction reactions (Phase I), the products of which are then available for conjugation reactions (Phase II) and excretion. The most important Phase I enzymes are the cytochrome P450s which metabolise toxins, but also activate some carcinogens, so that induction can lead to enhanced carcinogenic activity. Phase II enzymes such as glutathione-S-transferase (EC 2.5.1.18) and UDP-glucuronyl transferase form conjugation products which are more polar than the unconjugated species and hence more readily excreted, so their induction is considered to be wholly protective.

There is now ample evidence from animal models to show that certain isothiocyanates and their conjugates can inhibit the cytochrome p450 enzymes which activate nitrosamines to alkylating carcinogens responsible for induction of lung tumours.37,38

The most important block inhibition appears to be achieved by induction of enzymes of Phase II of the biotransformation system, which involve conjugation with glucuronic acid or glutathione. These reactions render the metabolites more polar and hence more readily excreted. The induction of such Phase II enzymes has been shown to occur after treatment with isothiocyanates, and this may be responsible for the observed protection against induction of tumours.

### Table 1. Glucosinolate levels of freeze-dried Brassica vegetables (μmol g⁻¹ dryweight)

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Aliphatic/aromatic glucosinolates</th>
<th>Indolyl glucosinolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 Total</td>
<td>10 11 12 13 Total 1–13</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0.2 – 0.2 – 0.3 – – – – –</td>
<td>0.7 0.1 0.7 0.2 0.1 1.1 1.8</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>3.1 7.6 8.2 2.1 0.3 7.0 0.3 – –</td>
<td>28.3 1.2 4.5 0.9 – 6.6 34.9</td>
</tr>
<tr>
<td>Savoy cabbage</td>
<td>4.3 0.3 4.3 0.4 0.3 0.4 – –</td>
<td>10.0 0.4 2.4 1.8 0.1 4.7 14.7</td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.5 6.2 – 5.9 0.3 0.6 – –</td>
<td>13.5 – 2.1 0.2 0.9 3.2 16.7</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>1.6 1.2 2.7 0.3 0.1 0.4 – –</td>
<td>6.2 0.1 3.8 0.3 – 4.2 10.4</td>
</tr>
<tr>
<td>Green cabbage</td>
<td>7.3 0.2 10.2 – 0.2 – –</td>
<td>17.9 – 6.8 1.3 – 8.1 26.0</td>
</tr>
<tr>
<td>Oxheart cabbage</td>
<td>0.7 0.3 0.1 0.9 0.3 – –</td>
<td>2.3 0.1 1.1 0.1 – 1.3 3.6</td>
</tr>
<tr>
<td>White cabbage</td>
<td>6.8 0.2 4.2 0.1 0.2 – –</td>
<td>11.5 – 3.4 0.4 0.1 3.9 15.4</td>
</tr>
<tr>
<td>Kohlrabi</td>
<td>0.2 – – 0.2 0.1 – – –</td>
<td>0.5 0.1 1.3 0.1 0.5 2.0 2.5</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>– – – – – – 0.5 –</td>
<td>0.5 – 1.3 1.5 0.1 2.9 3.4</td>
</tr>
<tr>
<td>Swede</td>
<td>– 2.3 – – 0.7 – 2.9 – 0.5</td>
<td>6.4 0.2 0.9 0.3 0.9 2.3 8.7</td>
</tr>
<tr>
<td>Radish</td>
<td>– 0.2 – – 0.2 – 4.9 0.7</td>
<td>6.0 0.2 0.3 0.5 – 1.0 7.0</td>
</tr>
<tr>
<td>Horseradish</td>
<td>– – – – – 0.2 – 9.6 0.4</td>
<td>10.2 0.3 0.3 0.1 – 0.7 10.9</td>
</tr>
</tbody>
</table>

1=glucobrassicin; 2=progoitrin; 3=sinigrin; 4=glucobrassicanil; 5=glucoraphanin; 6=glucopropin; 7=glucosturtiin; 8=4-methylthiobutyleucinoglucosinolate; 9=rest; 10=4-hydroxyglucobrassicin; 11=glucobrassicin; 12=4-methoxyglucobrassicin; 13=neoglucobrassicin.

From Tiedink et al, Ref. 52.
Phenethyl isothiocyanate (PEITC) in particular has been shown to inhibit the action of the rodent lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), probably by blocking its activation and increasing urinary excretion of its metabolites.\(^{39,40}\) PEITC is a derivative of the glucosinolate gluconasturtiin, which occurs in large quantities in watercress. In one of the few published studies on the anticarcinogenic activity of glucosinolates in humans, consumption of 57g of watercress at each meal for three days led to a substantial increase in the excretion of NNK metabolites in human smokers.\(^{41}\) There has also been much recent interest in isothiocyanates which can exert blocking effects by induction of Phase II enzymes.\(^{42}\) An important example is sulphoraphane, an isothiocyanate present at high levels in broccoli, which induces Phase II enzymes \textit{in vitro},\(^{43}\) but other isothiocyanates derived from common brassica vegetables may well exert comparable levels of biological activity.\(^{39}\)

By definition, any mechanism of tumour suppression must involve complex interactions between protective substances or their metabolites and nascent tumour cells, at some stage after the initial induction of unrepaired DNA damage. Suppressing mechanisms are probably more numerous than blocking mechanisms, but they are much less well characterised.\(^{3}\) It is likely that inhibition of cell proliferation, induction of cell differentiation and an increased rate of programmed cell death (apoptosis) in irretrievably damaged cells are all involved. Recent evidence suggests that glucosinolate breakdown products might be involved in the latter mechanism. Allyl isothiocyanate is selectively cytotoxic to cancer cells \textit{in vitro},\(^{44,45}\) and it been established that phenethyl isothiocyanate induces cell death via an apoptotic pathway.\(^{46,47}\) Moreover Smith \textit{et al}\(^{48}\) have shown that oral administration of sinigrin suppresses proliferation and increases apoptosis in the basal regions of colorectal crypts in rats, but only in animals previously treated with the colonic carcinogen dimethylhydrazine (DMH). This effect was associated with a significant reduction in the numbers of precancerous mucosal lesions (aberrant crypt foci) induced by the DMH treatment. Further studies are needed to determine the relative importance of blocking and suppressing effects in the prevention of human cancer.

**PLANT FOOD SOURCES**

Glucosinolates occur only in dicotyledonous angiosperms, and they are prevalent in the Brassicas, which are widely cultivated as vegetables, seasonings, condiments and sources of oil and feed.\(^{49}\) Phenylethyl glucosinolate is found at high levels in some minor crops such as radishes and watercress, and hydroxybenzyl glucosinolates are the major component of white mustard, \textit{Sinapis alba}. Generally, each plant species contains up to four different glucosinolates in significant amounts. The highest concentrations are usually found in the seeds, except for indol-3-ylmethyl and N-methoxyindol-3-ylmethyl glucosinolates, which are rarely found in seeds.\(^{50}\) Several published studies have described the glucosinolate composition of cabbages and rapeseed varieties, and these have been reviewed extensively by Fenwick \textit{et al}\(^{5}\) and more recently by Rosa \textit{et al}.\(^{51}\) Large differences in the levels of both methionine-derived glucosinolates and indolyl glucosinolates derived from tryptophan are observed in food plants, even within the same studies, but the differences are even greater when different studies are compared. Possible explanations for such variation are the use of different varieties, growing conditions and analytical methods. One example of a study on the levels of glucosinolates in different Brassica vegetables is given in Table 1.\(^{52}\)
BIOSYNTHESIS AND DEGRADATION OF GLUCOSINOLATES

Glucosinolate biosynthesis can be considered in three stages (1) amino acid chain elongation, (2) synthesis of the glucosinolate from the amino acid and (3) chain modifications (See Fig 2). However, for each of these stages relatively few enzymes have been characterised or genes cloned, and several steps of the biochemical pathway must remain speculative. The biosynthetic pathway for glucosinolates is the subject of two recent reviews, where further details can be found.

Side-chain elongation

Many glucosinolates are synthesised from chain-elongated forms of valine, phenylalanine and methionine (Fig 1). Biochemical studies involving the administering of 14C-labelled acetate and 14C labelled amino acids to plants, and subsequent analysis of the labelled glucosinolates, suggest that amino acid elongation is similar to the synthesis of leucine from valine and acetate. The amino acid is transaminated to produce an α-keto acid, followed by condensation with acetyl CoA, followed by isomerisation involving a shift in the hydroxyl group and oxidation to result in an elongated keto acid which is transaminated to form the elongated amino acid (Fig 3). It is likely that the elongated keto acid can undergo further condensations with acetyl CoA to result in multiple chain elongations. These are particularly characteristic of glucosinolates derived from methionine in which up to nine methylene groups may be added (Fig 1). There has been relatively little attempt to purify enzymes from this part of the biosynthetic pathway. A potential methionine aminotransferase has been partially purified from B carinata, but its involvement in glucosinolate biosynthesis has not established. However, the enzymes and genes involved in leucine biosynthesis have been well characterised from several microorganisms and some plants, and it is likely that genes involved in amino acid elongation will show significant homology to some of these.

Glucose biosynthesis

The first step in glucosinolate biosynthesis is the conversion of the amino acid to an oxime (Fig 4). Several studies have demonstrated that different enzymes are involved in the conversion of different amino acids. These studies have used mainly isolated microsomes as experimental systems. The best characterised of the enzymes are cytochrome P450 monooxygenases which convert tyrosine and phenylalanine to their corresponding oximes. Interestingly, similar enzymes convert these amino acids to oximes as the initial steps of cyanogenic glycoside biosynthesis. The conversion of chain-elongated forms of methionine and phenylalanine is catalysed by flavin-containing monooxygenases while the conversion of tryptophan to indole acetaldoxime, as the precursor of indole glucosinolates, is mediated by plasma membrane bound peroxidases. The specificity for each of these enzymes for the particular amino acid substrate may provide one point in the biosynthetic pathway where different classes of glucosinolates could be independently regulated, and is thus of interest to genetic modification approaches which may seek to up or down regulate specific glucosinolates.

The intermediates between the oximes and the thiohydroximates have not been identified (Fig 4), and this part of the pathways must be considered speculative. It has been proposed that the oxime is oxidised to an aci-nitro compound which then conjugates with cysteine which functions as the thiol donor. This reaction may be catalysed by a glutathione-S-transferase. The resulting S-alkylthiohydroximate may be cleaved by a CS-lyase to yield the thiohydroximate. The final steps in the biosynthesis of the glucose are the most fully understood. The thiohydroximate is S-
glucosylated by a soluble UDPG:thiohydroximate glucosyltransferase (S-GT) to produce a desulphoglucosinolate.70–72 This is sulphated by a soluble 3' phosphoadenosine 5'phosphosulphate (PAPS): desulphoglucosinolate sulphotransferase.73,74 Both of these enzymes have been partially purified from several species. In B juncea, the enzymes involved in sequential glycosylation and sulphation co-purify, suggesting that they may exist as an enzyme complex.75 Characterisation of S-GT from B oleracea has shown that it has high substrate affinity for thiohydroximates, but little specificity for side chain structure. Sulphotransferases have been purified from Lepidium and B juncea.73,74 As with S-GT, the enzymes were able to catalyse the sulphation of several different desulphoglucosinolates.

**Side-chain modification**

Following the biosynthesis of methylthioalkyl glucosinolates (or possibly the desulphoglucosinolate or thiohydroximate) from methionine, the side-chain may undergo various modifications. The suggested pathway involves an initial oxidation to methylsulphinylalkyl (and probably, in some species, methylsulphonylalkyl glucosinolates), followed by the removal of the methylsulphinyl group and desaturation to result in alkenyl glucosinolates, and subsequent hydroxylation to give hydroxalkenyl glucosinolates.76–78 However, within this part of the pathway there is considerable scope for variation. For example, 4-methylsulphinylbutenyl glucosinolate found exclusively in Raphanus probably results from the desaturation of the corresponding methylsulphinylbutyl glucosinolate but without associated methylsulphinyltransferase activity, whereas, 3-hydroxypropyl and 4-hydroxybutyl glucosinolates found in A thaliana probably result from the removal of a terminal methylsulphinyl group and hydroxylation as opposed to desaturation. The biochemistry underlying these modifications has received relatively little attention, except for the final hydroxylation of 3-butenyl glucosinolate which has been shown to be catalysed by a cytochrome P450 hydroxylase.77 Other modifications also occur of which very little is known. For example, benzoyloxyalkyl glucosinolates are found in the seeds of A thaliana and presumably arise from the conjugation of hydroxalkyl glucosinolate with benzoic acid. Side chains of glucosinolates derived from branched-chain amino acids and phenylalanine can also be hydroxylated, whereas as those from tryptophan frequently have methoxy groups added (Fig 1).

**Glucosinolate degradation**

The localisation and degradation of glucosinolates have been recently reviewed.79 Histochemical and immunological studies have shown that myrosinase is located in the cytoplasm of specialised myrosin cells scattered throughout the plant tissue and may also be located in the cytoplasm of other cells.80,81 It has been speculated that glucosinolates may be located in the cell vacuole,80,82 although definitive evidence is lacking. When tissue disruption occurs, myrosinase activity results in the cleavage of the glucose to leave an unstable intermediate. This aglycone spontaneously rearranges to produce several products (Fig 5a). Most frequently, it undergoes a Lossen rearrangement to produce an isothiocyanate. If the isothiocyanate contains a double bond, and in the presence of a epithiospecifier protein, the isothiocyanate may rearrange to produce an epithionitrile (Fig 5b).83 At lower pH, the unstable intermediate may be converted

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**Figure 5.** (a) Generalised scheme of glucosinolate hydrolysis. (b) Isothiocyanates with an alkenyl side-chain, for example 3-butenyl glucosinolate, can rearrange to form epithionitriles. (c) Isothiocyanates with a β-hydroxylated side-chain, for example 2-hydroxy-3-butenyl glucosinolate, spontaneously cyclise to form the corresponding oxazolidine-2-thione. (d) Indolyl glucosinolates also form unstable isothiocyanates, which degrade to the corresponding alcohol and may condense to form diindolylmethane. At more acidic pH, indolyl glucosinolates can form indolyl-3-acetonitrile and elemental sulphur.
directly to a nitrile with the loss of sulphur. Conversion to nitriles is also enhanced in the presence of ferrous ions. Aglucones from glucosinolates which contain \(\beta\)-hydroxylated side-chains, such as 2-hydroxy-3-butenyl (‘progoitrin’) found in the seeds of oilseed rape, spontaneously cyclise to form the corresponding oxazolidine-2-thiones (Fig 5c). Indolyl glucosinolates also form unstable isothiocyanates, which degrade to the corresponding alcohol and may condense to form diindolylmethane. At more acidic pH, indolyl glucosinolates can form indolyl-3-acetonitrile and elemental sulphur (Fig 5d). This nitrile has auxin activity, and can also be converted to indole-3-acetic acid.

The chemical structure of the glucosinolate products is important for their biological activity. Small changes to side-chain structures can have significant effects. For example, while methylthioalkyl glucosinolates produce volatile and pungent isothiocyanates (the major flavour compound in salad rocket is 4-methylthiobutyl isothiocyanate), methylsulphinylalkyl glucosinolates (the next products in the biochemical pathway) produce non-volatile isothiocyanates with relatively mild flavours, such as those found in broccoli. Removal of the methylsulphinyl group and the addition of a double bond results again in a volatile isothiocyanate. Finally, addition of a hydroxyl group to 3-butenyl and 4-pentenyl glucosinolates results in the spontaneous cyclisation of the unstable isothiocyanate and the production of a non-volatile product.

**DISTRIBUTION AND GENETIC VARIATION OF GLUCOSINOLATES IN FOOD AND NON-FOOD PLANTS**

Glucosinolates are found in 16 dicotyledenous plant families. Molecular phylogenetic studies based upon DNA sequencing of the chloroplast \(rbcL\) gene and the nuclear 18S ribosomal RNA gene, combined with morphological and biochemical analysis, suggests that all glucosinolate-containing taxa, with the exception of the genus *Drypetes* of the Euphorbiaceae, have a monophyletic origin and can be included in an expanded Capparales.83 Thus it is likely that the glucosinolate/myrosinase system arose independently on two occasions, with one of these origins giving rise to the majority of glucosinolate-containing taxa.82

The nature and diversity of glucosinolates within the 15 families of the major clade is quite different. All families contain at least some genera which have benzyl or hydroxybenzyl glucosinolates derived from phenylalanine and/or tyrosine. They also all probably contain glucosinolates derived from branched-chain glucosinolates such as valine and leucine, although data are lacking for several taxa. Chain-elongated glucosinolates from phenylalanine are only found in the Resedaceae, Capparaceae and Brassicaceae, and the chemically diverse chain-elongated glucosinolates from methionine (which comprise approximately one-third of all known glucosinolates) are found only in the Brassicaceae, although a small number may also occur in the Capparaceae. Thus the chain-elongated glucosinolates appear to have evolved relatively recently.

Fenwick et al provide a comprehensive review of the glucosinolate content of crop plants. By far the most important glucosinolates are methionine-derived glucosinolates which are found in *Brassica* vegetables. In *B rapa* (Chinese cabbage and turnips) there is relatively little variation in structure. All genotypes, including wild accessions, accumulate some or all of 3-butenyl, 4-pentenyl glucosinolates and their hydroxylated forms. In contrast, there is considerable variation in *B oleracea*. For example, genotypes can be found which have methylthioalkyl, methylsulphinylalkyl, alkenyl or hydroxyalkenyl glucosinolates, in which the alkyl chain has either three or four methyl groups. Moreover, there is considerable variation in total amount of glucosinolates. For example, some horticultural forms have relatively low levels, while some wild forms have maybe a thousand-fold greater content. The prevalence of methionine-derived glucosinolates in crop species is fortunately also found in *Arabidopsis thaliana*, which has become the ‘model’ species for plant geneticists and molecular biologists.

**GENETICS OF ALIPHATIC GLUCOSINOLATES IN *BRASSICA* AND *ARABIDOPSIS***

The majority of genetic studies on glucosinolate biosynthesis have focused on the methionine-derived glucosinolates occurring in *Brassica* and *Arabidopsis*. Most attention has been paid to reducing the glucosinolate content of seeds of oilseed rape (*B napus*). This has been successfully achieved through the introgression of genes from the low-seed glucosinolate cultivar Bronowski into initially Canadian spring rape cultivars and then European winter rape cultivars. The reduction has only been in methionine-derived glucosinolates, with no effect on the level of glucosinolates derived from tryptophan or phenylalanine. Through the use of *B napus* linkage maps and double haploid lines, a small number of quantitative trait loci (QTLs) which regulate the levels of glucosinolates have been positioned within the *B napus* genome.84,85 This genome is complex, and restriction fragment length polymorphism (RFLP) loci identified through the use of a single DNA probe were associated with several of the QTLs, suggesting that the underlying genes for each of these QTLs may be copies of each other. Comparative mapping studies have also suggested that QTLs in the *B oleracea* and *Arabidopsis* genomes which regulate glucosinolate content may be homologues of the same genes. Whether these genes are structural genes which code for particular enzymes in the biosynthetic pathway, or whether they are transcription factors which modulate the activity of a series of genes is not known at present.

A series of Mendelian genes have been mapped which regulate the elongation of the glucosinolate side chain.10 This has been achieved through the use of genotypes of *A thaliana* and *B oleracea* which have
different types of glucosinolates. For example, in *A. thaliana*, alleles at a single locus on chromosome 5 regulate whether the aliphatic glucosinolate has a three-carbon side-chain due to the addition of a single methylene to methionine, such as 3-hydroxypropyl glucosinolate found in the ecotype *Landsberg erecta*, or a four-carbon side-chain due to the addition of two methylene groups to methionine, such as 4-methylsulphinylbutyl glucosinolate found in the ecotype *Colombia*. Homologous loci have been mapped in *Brassica*. Loci have also been mapped which regulate side-chain modification. In *A. thaliana*, alleles at a single locus on chromosome 4 regulate the conversion of methylsulphinylpropyl glucosinolate to 3-hydroxypropyl glucosinolate in some ecotypes, and other alleles at this same locus convert this glucosinolate to 2-propenyl glucosinolate in other ecotypes. Again, homologous genes have been mapped in *Brassica*.

**Plant breeding**

Conventional plant breeding has been successful in making changes to the total amount of glucosinolates and to side-chain structure. The best known example is, as described above, the reduction in seed glucosinolate content in oilseed *B. napus*. However, more subtle changes have also been made. For example, in an effort to deter pigeons and slugs from oilseed rape crops, the level of 3-butenyl glucosinolate has been specifically enhanced through the introduction of null alleles at the *Gsl-oh* loci (Fig 2), which prevent the addition of hydroxyl groups, and the production of a null allele at an elongation locus which prevents the synthesis of 4-pentenyl glucosinolates. Wild *Brassica* species which accumulate high levels of certain glucosinolates are being used to enhance the level of the potential anticarcinogenic methylsulphinylalkyl glucosinolates in broccoli.

**Genetic modification**

Considerable effort is now focused on cloning genes and the genetic modification of the total level and side-chain structure of glucosinolates. The cloned S-GT gene is a good candidate to use to attempt to down-regulate total glucosinolate biosynthesis due to the apparent low specificity of the S-GT for different glucosinolates. It is likely that several genes will soon be cloned from *A. thaliana* which will be used to modify the glucosinolate content of *Brassica*. However, it may also be possible to reduce glucosinolate content by diverting the flow of substrates away from glucosinolate biosynthesis. For example, redirection of tryptophan has led to a reduction of indole glucosinolate content and expression of a mammalian metallothionen has resulted in suppression of glucosinolate biosynthesis. In addition to altering levels, it is likely that differential spatial and temporal regulation of glucosinolates will be sought through the use of different promoters.

With regard to the beneficial effects of glucosinolates, it may be possible to enhance specific glucosinolates. For example, down-regulating the *ALK* gene (Fig 2) which converts methylsulphinylalkyl glucosinolates to alkenyl glucosinolates in horticultural brassicas would be expected to have a beneficial effect on the induction of Phase II enzymes. Increasing total glucosinolate content may require the cloning of regulatory factors for which, at present, we lack information.

**ANALYTICAL METHODS**

The abundance and structural variety of the glucosinolates, and the fact that each can produce different breakdown products makes their analysis very complicated. The analytical methods available have been extensively reviewed by McGregor *et al* and more recently by Verkerk *et al*, and only a brief overview is presented here.

Because glucosinolates coexist with myrosinase in the plant, processes like grinding or cutting of fresh tissue in the presence of water will initiate a rapid hydrolysis of the parent compounds, and this adds greatly to the complexity of the problem. In general the analytical approach can be divided into methods for total glucosinolates, individual glucosinolates and the breakdown products. For analysis of intact glucosinolates inhibition of myrosinase activity is essential. Before disruption of the material, samples should be completely dried by freeze-drying or frozen in liquid nitrogen. The use of aqueous methanol for extraction, in combination with high temperatures, also inhibits myrosinase.

**Total glucosinolates**

Glucosinolates yield equimolar amounts of glucose upon hydrolysis with myrosinase, and methods based on the measurement of enzymatically released glucose proved to be relatively rapid and simple to apply. The total glucosinolate content of a food sample can be measured by determining the quantity of glucose released after treatment with the enzyme, but account must be taken of any endogenous glucose. To achieve this, extraction of glucosinolates can be performed followed by selective cleanup that eliminates free glucose and other interfering compounds, after which controlled enzymatic release of bound glucose is possible.

As mentioned earlier, myrosinase hydrolysis of glucosinolates gives rise to an unstable aglucone, which after a Lossen rearrangement produces an equimolar quantity of bisulfate. Several methods have been described for the quantification of this bisulfate ion using titrimetric and gravimetric methods. Schnug has described a method in which the bisulfate liberated after sulfation is precipitated with barium chloride, and residual barium is measured by X-ray emission spectroscopy.

**Individual glucosinolates**

Gas liquid chromatography (GLC) of derivatised
Glucosinolates is the traditional method for the identification and quantification of the individual compounds. Originally the glucosinolates were extracted with boiling water, derivatised and separated by isothermal chromatography but substantial improvements have subsequently been made by Thies. In particular, ion exchange purification of glucosinate extracts to remove carbohydrates and other impurities before derivatisation has increased the sensitivity. Another major breakthrough in glucosinolate analysis has been achieved with the introduction of enzymatic on-column desulfation using aryl sulfatase. The introduction of a desulfation step before derivatisation was performed to eliminate sulfate that interfered with GC analysis. Desulfation was elegantly carried out on the ion exchange column, using a commercially available sulfatase isolated from an edible snail (Helix pomatia). Free sulfate in the glucosinolate extract, which could inhibit the sulfatase, was precipitated by addition of barium acetate and removed by centrifugation before addition of the extract to the ion exchange column.

Some glucosinolates, particularly the indoles, are thermally unstable and HPLC has therefore become the preferred method. High performance liquid chromatography (HPLC) has the advantage of direct determination of glucosinolates. The first successful application of the technique was described by Helboe et al. Glucosinolates were purified and desulfated on-column, and then separated by ion-exchange chromatography or reverse phase ion-pairing chromatography using a C18 Nucleosil column with gradient elution using acetonitrile-water mixtures as the mobile phase and tetracylammonium bromide as the source of counter ion. By avoiding the use of buffer solutions and ion-pairing reagents, glucosinolates could be collected in a pure form suitable for identification by mass spectrometry. With the aid of this method, two new glucosinolates have been separated and identified, 4-hydroxy-3-indolylmethyl glucosinolate and 4-methoxy-3-indolylmethyl glucosinolate.

One of the major problems in the analysis of glucosinolates has been the lack of suitable standards. The only commercial available glucosinolates are benzylglucosinolate (glucotropaeolin) and 2-propenylglucosinolate (sinigrin). Sinigrin is not a suitable internal standard because of the presence of this compound in most brassicaceous plants, but glucotropaeolin is not normally present in Brassica and has been used as internal standard. Several mass spectroscopic techniques have been investigated for structure elucidation of the various (desulpho-)glucosinolates eg direct probing electron impact, chemical ionisation, and fast atom bombardment. Considerable structural information can be obtained with these techniques.

Finally van Doorn has recently described a novel ELISA procedure for the determination of sinigrin and progoitrin in Brussels sprouts extracted with phosphoric acid, using antisera raised against hemisuccinate-linked glucosinolate conjugates. The method tended to overestimate glucosinolate content in comparison to HPLC methods but seems to offer great potential advantages in terms of the cost and time needed for routine analysis in breeding programmes.

Breakdown products

The application of HPLC to the investigation of glucosinolate breakdown products has been limited due to the volatility of many compounds. Furthermore, thiocyanates and nitriles are not detectable spectrometrically. Isothiocyanates and nitriles can be analysed by GLC. HPLC with UV detection may be used for analysis of oxazolidinethiones and indoles. Quinsac et al developed a method for analysing oxazolidinethiones in biological fluids with a high degree of selectivity. However HPLC finds most use in the analysis of intact glucosinolates or desulfoglucosinolates. For identification and confirmation of structures, both techniques can be coupled to mass spectrometry (MS). Mass spectroscopy has proved to be an invaluable tool in the identification and structural elucidation of glucosinolates and their breakdown products. Positive ion fast atom bombardment mass spectrometry (FAB) has yielded mass spectra characterised by abundant protonated and cationised molecular ions with relatively little fragmentation. In the negative ion mode, FAB produces an abundant molecular ion (of the glucosinolate anion). This proved especially advantageous in the analysis of crude plant extracts and mixtures of purified glucosinolates.

Zhang developed a spectroscopic quantitation of organic isothiocyanates. Under mild conditions nearly all organic isothiocyanates (R-NCS) react quantitatively with an excess of vicinal dithiols to give rise to five-membered cyclic condensation products with release of the corresponding free amines (RNH2). The method can be used to measure 1 nmol or less of pure isothiocyanates or isothiocyanates in crude mixtures.

**EFFECT OF PROCESSING**

Brassica vegetables are inevitably subjected to a variety of stresses after harvesting, and during processing and cooking prior to consumption. Stress-induced increases in levels of glucosinolates have been observed in response to mechanical wounding and infestation, methyl jasmonate exposure for intact plants, or UV irradiation for post-harvest vegetables. Processes like chopping for raw consumption, cooking and fermentation, damage plant cells and bring myrosinase in contact with glucosinolates. For reasons described earlier, such changes influence the levels of glucosinolates, the extent of hydrolysis, and therefore the composition, flavour and aroma of the final products. De Vos and Blijleven have previously reviewed the effects of processing on glucosinolate levels in vegetables.
Domestic processing and cooking
Before Brassica vegetables can be consumed they must usually be chopped up. Cutting the fresh plant tissues creates optimal conditions for myrosinase so that a high degree of glucosinolate hydrolysis can be expected, and in the extreme case, pulping of plant tissues results in the complete breakdown of glucosinolates by autolysis. However, Verkerk et al. observed elevated levels of all indolyl glucosinolates and some aliphatic after chopping and prolonged exposure to air of different kinds of Brassica vegetables. In white cabbage the largest increase they found was for 4-methoxyglucobrassicin which increased 15-fold. Apparently, chopping of post-harvest Brassica crops induces physiological changes which markedly affect the levels of individual glucosinolates. The total glucosinolate content of processed cabbage is possibly a reflection of two opposing mechanisms, namely breakdown of glucosinolates by myrosinase, and formation of especially indolyl glucosinolates caused by an unknown mechanism. Just as with induction of indolyl glucosinolates by infestation or mechanical wounding of Brassica plants, increasing the amount of glucosinolates by infestation or mechanical wounding of Brassica plants, increasing the amount of indolyl glucosinolates during vegetable processing could have large influences on quality factors such as flavour and anticarcinogenicity.

The effect of cooking on glucosinolates has received a relatively large amount of attention. Cooking reduces glucosinolate levels by approximately 30–60%, depending on the method (eg conventional, microwave, high pressure), cooking intensity (eg temperature, time), and on the type of compound. Also thermal degradation and wash-out occur, leading to large losses of intact glucosinolates. Glucosinolate breakdown products are apparently hardly detectable after prolonged cooking, with the exception of the thiocyanate ion and ascorbigen.

Industrial processing
The mechanisms that affect the levels of glucosinolates and breakdown products in Brassica vegetables processed in the home will also play a role in industrial processing. The main food products produced commercially from Brassica vegetables are washed, pre-cut cabbage: (processing steps: washing, cutting, packaging), fermented cabbages (processing steps: washing, cutting, fermentation, packaging) and frozen sprouts and cabbages (processing steps: washing, cutting, blanching/cooking, freezing, packaging). It should now be clear that cutting or slicing of the vegetable tissue will trigger complex reactions mechanisms changing the levels of glucosinolates and subsequently their breakdown products. However, as indicated earlier, glucosinolate levels do not necessarily decline rapidly after chopping and even induction can take place. Nevertheless when the sliced vegetables are washed as part of the processing, the conditions for myrosinase activity are probably optimal.

During the fermentation of white cabbage to produce sauerkraut, all glucosinolates were hydrolysed within two weeks according to Daxenbichler et al. The breakdown products investigated were the thiocyanate ion, isothiocyanates, goitrin and the nitriles 1-cyano-3-methylsulfinylpropane (from glucoiberin) and 1-cyano-2.3-epithiopropane (from sinigrin). Isothiocyanates, goitrin, and cyano-2.3-epithiopropane were not detectable throughout fermentation.

Wathelet et al. investigated the effect of blanching on the quality of Brussels sprouts. His results showed that blanching before freezing induces chemical enzymic and physicochemical modifications. However blanched Brussels sprouts did not exhibit a significant reduction of glucosinolates. Also the myrosinase activity was reduced when treatment time and temperature increased. Goodrich et al. made similar observations when comparing different blanching conditions for Brussels sprouts and broccoli. Their study indicated that large glucosinolate losses occur in blanched broccoli, but not in blanched Brussels sprouts. The tight, compact sprouts appeared to be more resistant to the leaching effects of water or steam blanching as compared to the loose structure of the broccoli stalk and flower heads.

Low-temperature storage like freezing and refrigerating can alter the metabolism of glucosinolates. Freezing without previous inactivation of myrosinase results in almost complete glucosinolate decomposition after thawing. There are no systematic studies on the changes in glucosinolate levels under packed conditions. It would be most interesting to know the effects of packaging and storage on the levels in washed/pre-cut cabbages. Depending on the conditions (eg temperature, humidity and O₂, CO₂ concentrations) inside the package, the levels could either be decreasing as a result of myrosinase activity or increasing as a result of induction as mentioned earlier. More research is required to confirm this hypothesis and to predict which mechanism will be dominating under specific conditions.

Storage
Although Brussels sprouts, cauliflower and broccoli are not storable for more than a few weeks, many types of cabbage are stored for long periods at low temperatures. Storage of heads of white and red cabbage for up to five months at 4 °C does not seem to affect the levels of glucosinolates (Verkerk, unpublished results), but in general there is still little information about the influence of storage on total or individual glucosinolate content of Brassica vegetables.

BIOAVAILABILITY
Clearly an understanding of the bioavailability, transport and metabolism of glucosinolates after consumption of Brassica vegetables as food is a prerequisite for understanding the mechanisms of their protective effects in humans. The structural diversity and chemical reactivity of glucosinolate breakdown products, as well as the complexities of the milieu from...
which they have to be isolated, have long inhibited progress in this field but the improvements in analytical methods for detecting and quantifying isothiocyanates and their excretory metabolites are now transforming this situation.

**Metabolism in the gastrointestinal tract**

Current evidence suggests that when plant myrosinase is present in the diet, glucosinolates are rapidly hydrolysed in the proximal gut (Campbell et al pers commun). If myrosinase is deactivated, for example by cooking the vegetables prior to consumption, the ionised nature of glucosinolates may be expected to enable them to reach the distal gut where they could be metabolised by bacterial enzymes. This hypothesis was first tested and confirmed by studies in which antibiotic treatments were used to reduce the large bowel microflora. More direct evidence was eventually obtained from gnotobiotic experiments in which the introduction of a whole faecal flora from rats or humans into initially germfree rats resulted in the disappearance of intact glucosinolates in the cecal and colonic contents, coupled with the emergence of systemic effects reflecting glucosinolate hydrolysis. It appears that the ability to degrade glucosinolates is widely distributed among intestinal bacteria, and Rabot et al have isolated from human faeces representative of various genera (e.g. Bacteroides, Peptostreptococcus, Enterococcus, Escherichia, Proteus) which are able to carry out the degradation of progoitrin and sinigrin in vitro.

As yet, little is known of the structure of microbial glucosinolate derivatives. Upon anaerobic incubation of cooked watercress juice with human faeces, 18% of glucosinolates are hydrolysed to isothiocyanates in 2h. The contribution of the digestive microflora to the production of isothiocyanates in vivo, in the distal gut, has been recently ascertained; following gavage with 50 μmol sinigrin, substantial amounts of allyl isothiocyanate (up to 100 nmol 12h after dosing) were measured in the cecal and colonic contents of gnotobiotic rats harbouring a human digestive strain of Bacteroides, while no allyl cyanide could be detected. It may be speculated that nitriles were converted nitriles to organic acids and ammonia.123 The formation of other derivatives, eg desulphoglucosinolates or thiocyanates, has scarcely been investigated, and studies are often not conclusive, chiefly because of analytical impediments. Nevertheless, the versatility of microbial enzymatic activities would be expected to lead to a wider array of metabolites than those so far identified.

Glucosinolate degradation also occurs to some extent in the upper digestive tract, probably by spontaneous chemical degradation, since no myrosinase activity has ever been found in digestive tissues. Maskell and Smithard attempted to simulate peptic and small intestinal digestion in pig under in vitro conditions and found that losses of individual glucosinolates ranged from 3 to 23% and 7 to 28% respectively, with indolyl glucosinolates being the most labile. Furthermore the acid environment of the stomach is known to convert indole-3-carbinol, a major hydrolysis derivative of glucobrassicin into a range of polycyclic aromatic condensation products. Ingested glucosinolates may also be partly absorbed, as indicated by reports of small amounts of intact glucosinolates in the blood and urine of poultry fed rapeseed meal. In an experiment designed to investigate the possibility of glucosinolate transport across intestinal mucosa, Michaelsen et al have shown that glucotropaeolin and, to a lesser extent, sinigrin were passively transported from mucosal to serosal side of everted sacs made from small intestine and colon of hamster.

**Post-absorptive metabolism and disposition**

Pharmacokinetic studies using orally administered isothiocyanates confirm their rapid absorption from the upper gastrointestinal tract. The rate of appearance of 14C in the blood after dosing rats with [14C]phenethyl isothiocyanate or [14C]allyl isothiocyanate (25 to 250 μmol kg-1) is rapid, with a peak concentration of 10 to 100 nmol ml-1 occurring at ~3h. Following absorption, the major route of isothiocyanate metabolism in humans is via their conversion to N-acetylcysteine derivatives (mercapturic acids or N-acetyl-S-(N-alkylthiocarbamoyl)-1-cysteine). This proceeds by initial conjugation with glutathione, promoted by glutathione-S-transferases, followed by hydrolysis of the resulting conjugates to the cysteine derivatives and final N-acetylation. Mennicke et al. found that 54% of a 1 μmol kg-1 dose of benzyl isothiocyanate given orally was excreted in urine as its N-acetylcysteine derivative within 24h. Studies with human subjects given appropriate foods have provided evidence for the renal excretion of the mercapturic acid of allyl isothiocyanate from brown mustard or horseradish, of phenethyl isothiocyanate from watercress and of sulphoraphane from broccoli homogenate after consumption of these foods; excretions were substantial, amounting to 30 to 67% of the ingested quantities within 24h. The same holds good for rat models which, upon oral exposure to allyl, benzyl or phenethyl isothiocyanate (25 to 250 μmol kg-1), or to sinigrin together with cauliflower as a source of myrosinase, excrete 35 to 75% of the ingested dose in the form of urinary N-acetylcysteine derivatives, mainly within 24h following the gavage. In contrast to these data, the recovery of mercapturic acids is substantially lower (≤20%), and the rate of excretion is slower (t1/2 ≤20h), when humans consume vegetables which have been cooked to inactive myrosinase or when rats are fed sinigrin in conjunction with diets free of


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myrosinase. These data clearly indicate that the overall extent and rate of disposition of isothiocyanates depends upon the contributions of plant and microbial myrosinase respectively, during the digestive conversion of glucosinolates.

Besides the mercapturic acid pathway, isothiocyanates may follow minor routes of metabolism. Rats dosed with \(^{14}\text{C}\)allyl isothiocyanate or \(^{14}\text{C}\)phenethyl isothiocyanate excrete a small fraction \((c.15\%\) of the radiolabel in the form of \(\text{CO}_2\) in exhaled air, and in faeces in the form of unknown metabolites. The higher value for biliary excretion suggests that some of the material undergoes enterohepatic circulation. In tissues, the greatest amounts of radioactivity are retained in the liver, kidneys and intestinal mucosa, followed by the lungs and spleen, with the brain and heart containing only very low levels.\(^{131,132,139}\) The greater proportion of radiolabel recovered in the faeces \((47\%)\) and in the exhaled air \((16\%)\) when rats are dosed with \(^{14}\text{C}\)6-phenethyl isothiocyanate, a synthetic homologue of phenethyl isothiocyanate, points to the possibility that the involvement of minor pathways, including oxidative metabolism and possible glucurononoconjugation in the liver and biliary excretion, may increase substantially, depending on the chemical features of the side-chain.\(^{132}\)

The post-absorptive fate of glucosinolate derivatives other than isothiocyanates has received comparatively little attention. Thiocyanates may be converted to cyanide and thiol derivatives by glutathione-\(S\)-transferases and epithionitriles may be excreted in the form of mercapturic acids.\(^{141}\) Thiocyanate ion is excreted almost unchanged in the urine but the published data regarding recovery levels are inconsistent.\(^{131,142}\)

**PRIORITY RESEARCH NEEDS**

As the genetic basis of glucosinolate biosynthesis becomes more apparent, and as tools for marker-assisted plant breeding become more available, it is possible to envisage the breeding by conventional means of a selection of horticultural brassicas with different levels and types of glucosinolates, particular in *E. oleracea* for which there is abundant genetic variation. Altering glucosinolates in *B. rapa* may require genetic modification with cloned genes, but it seems entirely probable that this will be feasible too. However, the marketing of such products will only be possible when and if the climate of consumer opinion becomes favourable. For this to occur, the health benefits of glucosinolates must be unequivocally established.

Whilst there is still much to be learnt from *in vitro* studies about the mechanisms of interaction between glucosinolate breakdown products and their target tissues, the major priority for future research must be *in vivo* studies with human volunteers. Such studies should preferably be conducted with chemically defined Brassica vegetables, and employ protocols which enable the dose-response relationship for both beneficial and adverse effects to be properly quantified. The major technical challenge will be the development of biomarkers to provide a comprehensive picture of the individual subject’s physiological response to dietary glucosinolates. It is now well known that glutathione-\(S\)-transferase isoenzymes vary considerably in their specificities for isothiocyanates since genes coding for these enzymes are polymorphic in humans, the overall metabolism of isothiocyanates in an individual may ultimately depend on their genotype. Fortunately the feasibility of exploring such issues is steadily increasing as sensitive new techniques for the measurement of changes in gene expression become available.

In conjunction with such studies, further investigations are needed to provide a comprehensive evaluation of glucosinolate bioavailability in the context of the whole diet. In particular, it must be stressed that many factors may influence the digestive and post-absorptive metabolism of glucosinolates and derivatives, and consequently their tissue disposition and excretion. For example, dietary fibre and minerals have been suspected to modulate the microbial metabolism of glucosinolates in the hindgut, and this points strongly to the role of the complex dietary environment as a determinant of the digestive fate of these compounds.

Finally, as with all phytochemicals, any exploitation of their beneficial effects depends upon a full understanding of their behaviour within the changing human food chain. The fate of glucosinolates in fresh materials during food production is extremely complex since it depends on several mechanisms of degradation and biosynthesis which seem to occur simultaneously. The development of a robust predictive model to quantify the effects of these phenomena, and the integration with it of models describing the bioavailability and biological activity of the most important glucosinolates in humans, should be the ultimate goal for future research in this area.

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