# GLUCOSINOLATES AND GLUCOSINOLATE DERIVATIVES: IMPLICATIONS FOR PROTECTION AGAINST CHEMICAL CARCINOGENESIS

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## GLUCOSINOLATES: OCCURRENCE AND METABOLIC FATE

### GLUCOSINOLATES IN THE PLANT

Glucosinolates (GSL) are sulphur-containing molecules produced from amino acids by the secondary metabolism of plants. Their occurrence is limited to some families of dicotyledonous angiosperms. Considering edible plants only, they occur predominantly in *Cruciferae* and *Capparideae* and, sporadically, in *Caricaceae* and *Tropaeolaceae* (Table 1).

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Table 1. *Glucosinolate-containing edible plants*

| | |
|---|---|
| *Cruciferae* | |
| *Brassica oleracea* L. | |
| *gongylodes* group | Kohlrabi |
| *capitata* group | Red/white cabbage |
| *sabaüda* group | Savoy cabbage |
| *gemmifera* group | Brussels sprouts |
| *italica* group | Broccoli |
| *botrytis* group | |
| var. *cauliflora* DC. | Cauliflower |
| var. *cymosa* Lam. | Calabrese (green sprouting broccoli) |
| *acephala* group | |
| var. *millecapitata* (Lev) Thell. | Thousand head kale |
| var. *medullosa* Thell. | Marrowstem kale |
| var. *selensia* | Curly kale |
| var. *sabellica* | Collard |
| *Brassica alboglabra* Bailey | Chinese kale |
| *Brassica pekinensis* (Lour.) Rupr. | Chinese cabbage (Pe-tsai) |
| *Brassica chinensis* L. | Chinese white cabbage (Pak-choi) |
| *Brassica campestris* L. | |
| ssp. *rapifera* (Metzg.) Sinsk. | Turnip |
| ssp. *oleifera* (Metzg.) Sinsk. | Turnip rape |
| *Brassica napus* L. | |
| var. *napobrassica* (L.) Peterm or ssp. *rapifera* (Metzg.) Sinsk. | Swede (Rutabaga) |
| var. *napus* | Winter, summer rape |
| *Brassica nigra* (L.L) Koch | Black mustard |
| *Brassica juncea* (L.) Czern et Coss | Brown mustard |
| *Brassica carinata* A. Br. | Abyssinian mustard (Ethiopian cabbage) |
| *Sinapis alba* L. | White mustard |
| *Crambe maritima* L. | Sea kale |
| *Raphanus sativus* L. | Radish |
| *Armoracia lapathifolia* Gilib | Horseradish |
| *Wasabi japonica* Matsum. | Wasabi (Japanese horseradish) |
| *Eruca sativa* (Miller) Thell. | Salad rocket |
| *Lepidium sativum* L. | Garden cress |
| *Nasturtium officinalis* R. Br. | Water cress |
| *Capparaceae* | |
| *Capparis spinosa* | Caper |
| *Caricaceae* | |
| *Carica papaya* L. | Papaya (Pawpaw) |
| *Tropaeolaceae* | |
| *Tropaeolum majus* L. | 'Nasturtium' (Indian cress) |

References are: Carlson *et al.* (1981), Fenwick *et al.* (1982), Carlson *et al.* (1987), Adams *et al.* (1989).

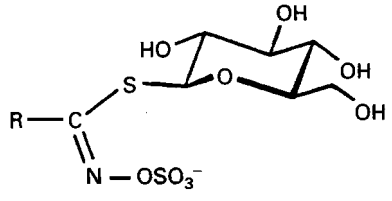


Fig. 1. The general structure of glucosinolates.

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Species belonging to these families are widely consumed by humans as cooked or salad vegetables (cabbage, Brussels sprouts, cauliflower, turnip, radish, cress) or condiments (horseradish, mustard, caper); cruciferous forages (kale, rape, turnip) and oilseed meals (rape, turnip rape) are used as feedstuffs for animals (Fenwick *et al.* 1982).

More than 100 different GSL, which all share a common structure (Fig. 1), have been identified so far (Fenwick *et al.* 1982). GSL may be classified into several chemical families according to their side groups R (Fenwick *et al.* 1986; Quinsac, 1993), which include alkyl, alkenyl, hydroxyalkyl, hydroxyalkenyl, methylthioalkyl, methylsulphinylalkyl, methylsulphonylalkyl, arylalkyl and indolyl groups (Table 2). Furthermore, a new family of GSL, designated cinnamoylGSL, was recently identified (Linscheid *et al.* 1980; Bjerg & Sørensen, 1987). It differs from the usual pattern by the presence of cinnamic acid derivatives in the C(2) and/or C(6) positions on the glucose moiety.

Edible plants may contain up to fifteen different GSL. However, most of them synthesize between one and five of these compounds. Concern about the potential biological effects of GSL has in the last decade prompted various groups to examine the levels and profiles of these compounds in cruciferous vegetables. The reader interested in detailed information is referred to the extensive research performed at the Northern Regional Research Center of the US Department of Agriculture (Daxenbichler *et al.* 1979; Carlson *et al.* 1981, 1985, 1987) and at the Norwich Laboratory of the Institute of Food Research in Britain (Heaney & Fenwick, 1980*a*, *b*; Fenwick *et al.* 1982; Sones *et al.* 1984*a*, *b*; Lewis & Fenwick, 1987, 1988). Findings published by these and other workers are schematically summarized in Table 3. On the whole, great variations in the content as well as in the pattern of GSL occur according to the plant species. The wide range of GSL concentrations sometimes observed within an experiment and between different studies performed on the same vegetable indicates that further variations may occur according to the cultivar and the cultivation conditions. Carlson *et al.* (1985) have pointed out the remarkable differences in the GSL content between radishes originating from either the European–American or the Asian market. Analysis of Brussels sprouts and cauliflower cultivars grown at different sites in the UK shows great variations in the total GSL content (Heaney & Fenwick, 1980*a*, *b*; Sones *et al.* 1984*b*); however, the relative proportions of the individual GSL tend to remain fairly stable within a cultivar. Climate, soil type and agronomic practices, especially fertilizer applications and harvest date, are cited as causative factors for such variations (Josefsson, 1970; Heaney & Fenwick, 1980*a*, *b*; Fenwick *et al.* 1982; Lehrmann, 1989; Booth *et al.* 1990).

Another factor of tremendous importance is the part of the plant examined. Major quantitative and qualitative differences in the GSL accumulated by different organs (seeds, leaves, roots) and different tissues of the same organ (root peelings, cortex and medulla) occur in the same plant (Heaney & Fenwick, 1980*a*, *b*; Sang *et al.* 1984; Carlson *et al.* 1987; Adams *et al.* 1989). Such findings highlight the point that GSL biosynthesis in the plant is probably ruled by complex control mechanisms and that one cannot extrapolate data available for one part of the plant to another tissue.

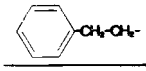
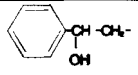
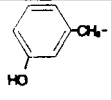
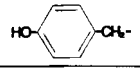
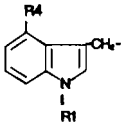
## GENESIS OF GLUCOSINOLATE DERIVATIVES

### *Enzymic hydrolysis and autolysis in cruciferous vegetables*

The breakdown of GSL by myrosinase, a specific plant hydrolytic enzyme (thioglucoside glucohydrolase *EC* 3.2.3.1), has been extensively studied and reviewed (Duncan & Milne, 1989).

In intact cruciferous tissues, the enzyme is stored separately from the GSL substrates in specific cells named idioblasts. Contact between the two will result from mechanical injury

Table 2. *Glucosinolates occurring in edible plants*

| Side chain | Glucosinolate | Trivial name |
|---|---|---|
| $CH_3$- | methyl- | glucocapparin |
| $CH_3$-$CH_2$- | ethyl- | glucolépidiin |
| $CH_3$-$CH(CH_3)$- | iso-propyl- | glucoputranjivin |
| $CH_3$-$CH_2$-$CH(CH_3)$- | 1-methylpropyl- | glucocochlearin |
| $CH_2$=CH-$CH_2$- | prop-2-enyl- | sinigrin |
| $CH_2$=CH-$CH_2$-$CH_2$- | but-3-enyl- | gluconapin |
| $CH_2$=CH-$CH_2$-$CH_2$-$CH_2$- | pent-4-enyl- | glucobrassicanapin |
| $CH_2$=CH-CH(OH)-$CH_2$- | (*R*)-2-hydroxybut-3-enyl-<br>(*S*)-2-hydroxybut-3-enyl- | progoitrin<br>epiprogoitrin |
| $CH_2$=CH-$CH_2$-CH(OH)-$CH_2$- | (*R*)-2-hydroxypent-4-enyl- | gluconapoleiferin |
| $CH_3$-S-$CH_2$-$CH_2$-$CH_2$- | 3-methylthiopropyl- | glucoiberverin |
| $CH_3$-S-$CH_2$-$CH_2$-$CH_2$-$CH_2$- | 4-methylthiobutyl- | glucoerucin |
| $CH_3$-S-CH=CH-$CH_2$-$CH_2$- | 4-methylthiobut-3-enyl- | glucoraphasatin |
| $CH_3$-S-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- | 5 -methylthiopentyl- | glucoberteroin |
| $CH_3$-SO-$CH_2$-$CH_2$-$CH_2$- | (*R*)-3-methylsulphinylpropyl- | glucoiberin |
| $CH_3$-SO-$CH_2$-$CH_2$-$CH_2$-$CH_2$- | (*R*)-4-methylsulphinylbutyl- | glucoraphanin |
| $CH_3$-SO-CH=CH-$CH_2$-$CH_2$- | (*R*)-4-methylsulphinylbut-3-enyl- | glucoraphenin |
| $CH_3$-SO-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- | (*R*)-5-methylsulphinylpentyl- | glucoalyssin |
| $CH_3$-$SO_2$-$CH_2$-$CH_2$-$CH_2$- | 3-methylsulphonylpropyl- | glucocheirolin |
| $CH_3$-$SO_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- | 4-methylsulphonylbutyl- | glucoerysolin |
|  | benzyl- | glucotropaeolin |
|  | 2-phenylethyl- | gluconasturtiin |
|  | (*R*)-2-hydroxy-2-phenylethyl-<br>(*S*)-2-hydroxy-2-phenylethyl- | glucobarbarin<br>glucosibarin |
|  | 3-hydroxybenzyl- | glucolepigramin |
|  | 4-hydroxybenzyl- | sinalbin |
|  | indol-3-ylmethyl- *(R1=R4=H)*<br>1-methoxyindol-3-ylmethyl- *(R1=$OCH_3$ ; R4=H)*<br>1-sulphoindol-3-ylmethyl- *(R1=$SO_3^-$ ; R4=H)*<br>4-hydroxyindol-3-ylmethyl- *(R1=H ; R4 = OH)*<br>4-methoxyindol-3-ylmethyl- *(R1=H ; R4 = $OCH_3$)* | glucobrassicin<br>neoglucobrassicin<br>sulphoglucobrassicin<br>4-hydroxyglucobrassicin<br>4-methoxyglucobrassicin |

References are: Fenwick *et al.* (1982), Quinsac (1993).

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Table 3. *Glucosinolate (GSL) content of the edible part of some cruciferous vegetables*

| Vegetable | Reference | GSL content (mg/100 g fresh weight) | | Major contributors (*a*) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | Indole GSL | | | |
| | | Mean | Range | SIN | GNA | GBN | PRO | GIV | GER | GRH | GIB | GRA | GRE | GAL | GST | GBS | NGBS | 4-OHGBS | 4-OMGBS |
| Kohlrabi | Carlson *et al.* (1987) | 39·4 | — | — | — | — | — | ✓ | ✓ | — | — | — | — | — | — | ✓ | — | — | — |
| Cabbage | Sang *et al.* (1984) | — | — | — | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | ✓ |
| | Sones *et al.* (1984*a*) | 108·9 | 36·0–275·4 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| | Slominski & Campbell (1989) | 51·0 | — | ✓ | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| Brussels sprouts | Heaney & Fenwick (1980*b*) | 199·0 | 90·0–390·0 | ✓ | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| | Sones *et al.* (1984*a*) | 226·2 | 145·5–393·9 | ✓ | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| | McMillan *et al.* (1986) | 247·0 | — | — | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| | Carlson *et al.* (1987) | 252·7 | 212·8–274·5 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| | Slominski & Campbell (1989) | 134·2 | — | ✓ | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| Broccoli | Carlson *et al.* (1987) | 86·0 | 46·7–121·0 | — | — | — | — | — | — | — | ✓ | ✓ | — | — | — | ✓ | — | — | — |
| | Slominski & Campbell (1989) | 27·6 | — | — | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | ✓ | — |
| Cauliflower | Sones *et al.* (1984*a*) | 62·0 | 13·8–208·3 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| | Sones *et al.* (1984*b*) | 78·3 | 26·0–214·2 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| | Carlson *et al.* (1987) | 43·2 | 18·8–73·4 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| | Slominsky & Campbell (1989) | 12·2 | — | ✓ | — | — | — | — | — | — | — | — | — | — | — | ✓ | — | ✓ | — |
| Calabrese | Lewis & Fenwick (1987) | 62·3 | 42·1–94·5 | — | — | — | — | — | — | — | — | ✓ | — | — | — | ✓ | ✓ | — | — |
| Kale | Carlson *et al.* (1987) | 144·0 | 29·4–317·1 | ✓ | — | — | — | — | — | — | ✓ | — | — | — | — | ✓ | — | — | — |
| Collard | Carlson *et al.* (1987) | 200·7 | 144·5–274·2 | ✓ | — | — | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| Chinese cabbage (pe-tsai) | Daxenbichler *et al.* (1979) | 54·1 | 17·4–135·7 | — | — | ✓ | — | — | — | — | — | — | — | ✓ | — | ✓ | ✓ | ✓ | ✓ |
| | Lewis & Fenwick (1988) | 19·8 | 9·7–33·7 | — | — | ✓ | ✓ | — | — | — | — | — | — | — | — | ✓ | — | — | — |
| Chinese cabbage (pak-choi) | Lewis & Fenwick (1988) | 53·4 | 39·0–70·4 | — | ✓ | ✓ | — | — | — | — | — | — | — | ✓ | — | — | — | — | — |
| Turnip | Carlson *et al.* (1981) | 125·1(*b*) | 98·7–230·8(*b*) | — | — | — | ✓ | — | — | — | — | — | — | — | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Sones *et al.* (1984*a*) | 56·0(*c*) | 39·2–165·7(*c*) | — | — | — | ✓ | — | — | — | — | — | — | — | ✓ | — | ✓ | — | — |
| Swede | Carlson *et al.* (1981) | 162·4(*b*) | 114·7–234·9(*b*) | — | — | — | ✓ | — | — | — | — | — | — | — | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Sang *et al.* (1984) | — | — | — | — | — | ✓ | — | — | — | — | — | — | — | — | — | ✓ | — | ✓ |
| | Adams *et al.* (1989) | — | — | — | — | — | ✓ | — | — | — | — | — | — | — | ✓ | — | ✓ | — | — |
| Mustard | Sang *et al.* (1984) | — | — | ✓ | — | — | — | — | — | — | — | — | — | — | — | — | — | ✓ | — |
| Radish | Sang *et al.* (1984) | — | — | — | — | — | — | — | — | ✓ | — | — | — | — | — | — | — | ✓ | ✓ |
| | Carlson *et al.* (1985) | 70·4(*d*) | 44·0–116·4(*d*) | — | — | — | — | — | — | ✓ | — | — | ✓ | — | — | ✓ | ✓ | ✓ | ✓ |
| | | 108·8(*e*) | 69·9–164·1(*e*) | — | — | — | — | — | — | ✓ | — | — | ✓ | — | — | ✓ | ✓ | ✓ | ✓ |
| | | 138·0(*f*) | 65·8–251·8(*f*) | — | — | — | — | — | — | ✓ | — | — | ✓ | — | — | ✓ | ✓ | ✓ | ✓ |

(*a*) SIN = sinigrin, GNA = gluconapin, GBN = glucobrassicanapin, PRO = progoitrin, GIV = glucoiberverin, GER = glucoerucin, GRH = glucoraphasatin, GIB = glucoiberin, GRA = glucoraphanin, GRE = glucoraphenin, GAL = glucoalyssin, GST = gluconasturtiin, GBS = glucobrassicin, NGBS = neoglucobrassicin, 4-OHGBS = 4-hydroxyglucobrassicin, 4-OMGBS = 4-methoxyglucobrassicin.

(*b*) peeled roots. (*c*) turnip/swede. (*d*) European–American market. (*e*) Korean market. (*f*) Japanese market.

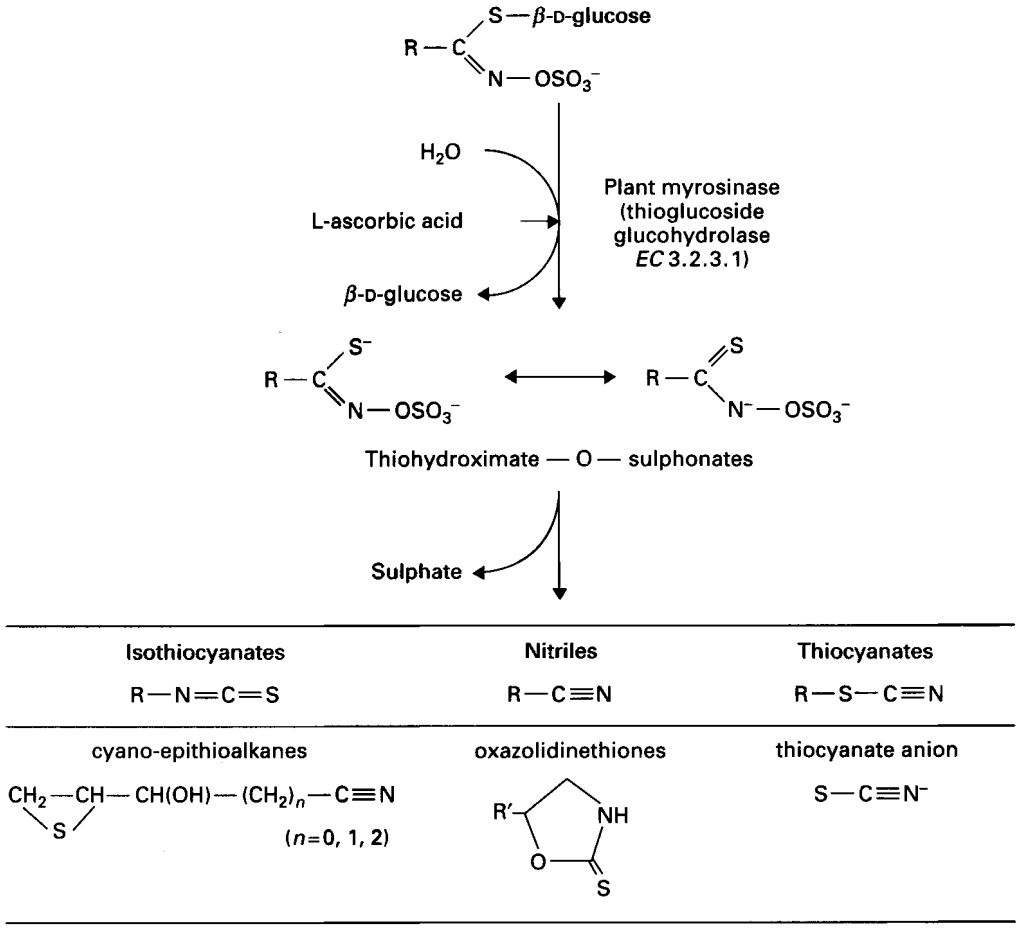


Fig. 2. The breakdown of glucosinolates by plant myrosinase.

of the plant tissue by, for example, cutting or chewing. Various isoenzymic forms of myrosinase have been isolated from different species and tissues (Fenwick *et al.* 1982; Buchwaldt *et al.* 1986). All of them hydrolyse the thioglucoside bond to release glucose and an unstable thiohydroximate-*O*-sulphonate, which is spontaneously further transformed (Lossen rearrangement; Ettlinger & Lundeen, 1956) to yield sulphate and a wide range of aglucones including isothiocyanates, nitriles, epithioalkanes, oxazolidinethiones, thiocyanate anions and, occasionally, organic thiocyanates (Fig. 2).

The enzymic step of the breakdown is usually enhanced by ascorbic acid, which acts as a specific coenzyme (Ettlinger *et al.* 1961; Ohtsuru & Hata, 1979). The structure of the aglucone eventually obtained is highly dependent on the structure of the side group R and on environmental factors such as pH, metallic ions ($Fe^{2+}$, $Fe^{3+}$, $Cu^{+}$ or $Cu^{2+}$) (Tookey & Wolff, 1970; Searle *et al.* 1984; Uda *et al.* 1986), and to a lesser extent temperature and moisture content (Tookey, 1973). For instance, low pH, low temperature or metallic ions will favour nitrile production, whereas neutral pH or high temperature will push the reaction toward isothiocyanate release (VanEtten *et al.* 1966; Gil & McLeod, 1980; Uda *et al.* 1986); the latter compound will tend to rearrange into oxazolidinethiones in an alkaline medium provided a hydroxyl group is present in the C(2) or C(3) position on the

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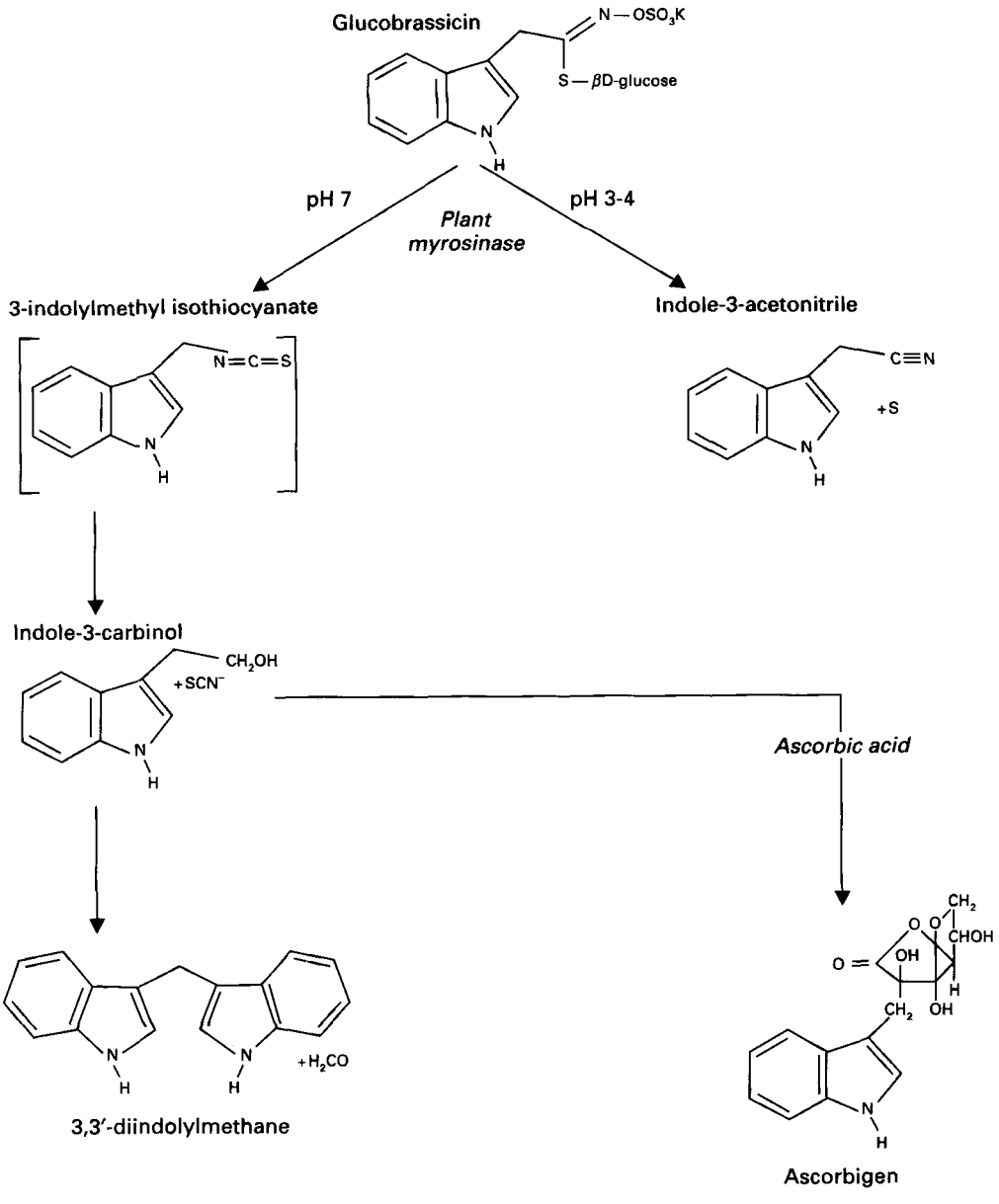


Fig. 3. The products of plant myrosinase hydrolysis of glucobrassicin.

side group R. Under the specific influence of ferrous ions autolysis can occur (Youngs & Perlin, 1967; Austin *et al.* 1968).

IndolylGSL may follow different patterns of enzymic breakdown. Fig. 3 summarizes the hydrolysis of glucobrassicin. Depending on the environmental conditions, glucobrassicin hydrolysis leads to the formation of indole-3-acetonitrile (IAN) (acidic pH, metallic ions) or of a putative unstable isothiocyanate derivative (neutral pH) which splits immediately to yield a thiocyanate anion and indole-3-carbinol (I3C). In the absence of ascorbic acid, two molecules of I3C condense to yield 3,3′-diindolylmethane. Should ascorbic acid be

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present in the medium, it will react with I3C to product ascorbigen (Searle *et al.* 1982). The glucobrassicin derivatives resulting from non-enzymic breakdown are different and vary with the pH: under acidic conditions, the first derivative would be IAN, thereafter transformed into indole-3-acetamide, indole-3-acetic acid and eventually skatole (minor product); a second pathway, which is less likely to occur, yields molecules such as indole-3-carboxaldehyde, indole-3-carboxylic acid and indole (McDanell *et al.* 1988). On the whole, the variety of compounds arising from the indolylGSL breakdown is greater than that of the aglucones derived from other GSL molecules.

*Bacterial metabolism of glucosinolates*

The observation that GSL derivatives could occur *in vivo* without prior ingestion of myrosinase prompted Greer and coworkers to look for myrosinase-like activity in body tissues and fluids. The exciting story of the investigations that led them to postulate that the human intestinal microflora was able to hydrolyse GSL *in vivo* is recorded in a review by Greer (1962).

An *in vitro* myrosinase-like activity in rat (Greer, 1962) and fowl (Marangos & Hill, 1974) faecal microflora was then demonstrated. Subsequently, different groups succeeded in isolating from human (Oginsky *et al.* 1965; Tani *et al.* 1974) and fowl (Miguchi *et al.* 1974) faecal microflora bacterial strains that were able to metabolize progoitrin or sinigrin *in vitro*.

Recent experiments performed with gnotobiotic animals in our laboratory at the Jouy-en-Josas Research Centre of the National Institute for Agricultural Research (INRA) in France definitely demonstrated that the myrosinase-like activity of the intestinal microflora was physiologically relevant since biological effects of cruciferous vegetables never occurred in germ free rodents and chickens given a GSL-rich but myrosinase free feed (Nugon-Baudon *et al.* 1988).

So far bacterial myrosinase-like activity has been considerably underinvestigated. Evidence for 5-vinyloxazolidine-2-thione (a progoitrin derivative) was assessed by Greer & Deeney (1959) in the urine of human volunteers after the ingestion of pure progoitrin, and by Oginsky *et al.* (1965) in the culture media of human Enterobacteriaceae strains incubated with the progoitrin. However, the breakdown of GSL by microflora is likely to be more complex than hydrolysis performed by the plant myrosinases. Experiments with gnotobiotic animals support the hypothesis that bacteria yield specific toxic GSL derivatives (Rabot *et al.* 1993*a*). Further conversion of several GSL derivatives into unknown compounds has been demonstrated in sheep rumen fluid *in vitro* (Lanzani *et al.* 1974; Duncan & Milne, 1992). There is little other information: the salient points in the studies of Oginsky *et al.* (1965) and Tani *et al.* (1974) are the influence of pH and the lack of influence, or even inhibitory effect, of ascorbic acid on bacterial myrosinase-like activities *in vitro*. *In vivo*, manipulation of the mineral (Vermorel & Evrard, 1987) or carbohydrate (Rabot *et al.* 1991) fraction of the diet helped to reduce the biological effects of cruciferous vegetables, implying that the bacterial myrosinase-like activities were altered in some way.

Improved and extended information on the myrosinase-like activities of the intestinal microflora would be of tremendous importance since plant myrosinase can be inactivated during processing of cruciferous vegetables; the implication is that a significant proportion of GSL must be actually metabolized by the intestinal microflora.

## FROM THE PLANT TO THE DIET: INFLUENCE OF FOOD PROCESSING AND DIETARY HABITS

When one knows the basic GSL content of cruciferous edible plants, it does not mean that one has reached the end of the story. Before being consumed, cruciferous vegetables usually undergo processing operations that may influence the GSL content. De Vos & Blijleven (1988) have extensively reviewed this subject and we report here only the main points relevant to the discussion in subsequent sections of the present review.

Basic processes such as dicing, slicing, or shredding raw vegetables initiate the breakdown of GSL by myrosinase, since rupture of tissues puts the enzyme into contact with its substrates. However, some intact GSL may remain, depending on the degree of crushing (de Vos & Blijleven, 1988). Pulping might of course be expected to result in a high degree of GSL breakdown. Indeed no intact GSL can be recovered from homogenized cabbage (de Vos & Blijleven, 1988) and Brussels sprouts (Bradfield & Bjeldanes, 1987) after 30 min and 24 h respectively. A preponderance of nitrile derivatives and, from indolylGSL, of ascorbigen and I3C have been identified, although the latter compound is not particularly stable and tends to undergo conversion into other products, mainly ascorbigen. Thus McDanell *et al.* (1987) have shown that the concentration of ascorbigen in Savoy cabbage homogenized to a thick slurry prior to deep-freezing and freeze-drying was 1·5 g/kg dry matter; this level represents 75% of the theoretical total of breakdown products, based on the glucobrassicin content.

Cooking, steaming and blanching usually reduce GSL concentrations by 30–60%, depending on the vegetable and on the type of GSL (Sones *et al.* 1984*a*); the loss is due partly to enzymic hydrolysis and partly to leaching of the intact GSL and their derivatives into the cooking liquid (Srisangnam *et al.* 1980*b*; Slominski & Campbell, 1989). The pattern of intact GSL and breakdown products recovered after cooking is influenced by the thermal stability of the molecules: sinigrin, for instance, is more thermostable than progoitrin or glucoiberverin, allyl isothiocyanate (from sinigrin) totally disappears upon boiling, while 5-vinyloxazolidine-2-thione (from progoitrin) and 3-methyl-sulphinylpropy-lisothiocyanate (from glucoiberin) may partly escape decomposition (de Vos & Blijleven, 1988). Once again, among glucobrassicin derivatives, ascorbigen appears to be the major compound recovered after cooking (McDanell *et al.* 1987).

Fermented cruciferous products (sauerkraut, salt fermented vegetables) contain no intact GSL since this kind of process favours their quick and complete enzymic hydrolysis. In a study by Daxenbichler *et al.* (1980), reported by de Vos & Blijleven (1988), the main GSL derivatives identified in sauerkraut after a 2 week fermentation were the thiocyanate anion and 1-cyano-3-methylsulphinylpropane (a nitrile from glucoiberin). However McDanell *et al.* (1987) found that fermentation (18 h, 25 °C) of white or Savoy cabbage was less detrimental to intact GSL than cooking; as far as GSL derivatives were concerned, the content of IAN was seldom modified by fermentation whereas there was an important decrease of ascorbigen compared with the fresh material.

Storage processes such as freezing, dehydrating or irradiating have received much less attention. From the few and often contradictory studies reported, one can conclude only that whereas dehydrating preserves intact GSL (de Vos & Blijleven, 1988), irradiation with u.v. or ionizing radiation tends to favour their breakdown (Michajlovskij, 1968 cited in McDanell *et al.* 1988; Nugon-Baudon *et al.* 1988; de Vos & Blijleven, 1988).

Table 4 reports the average consumption of cruciferous vegetables in several countries for which nutritional survey data are available. While cruciferous vegetables are consumed worldwide, this table highlights the fact that quantitative and qualitative differences occur between the geographical regions and/or the dietary habits characteristic of each country.

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Table 4. *Average weekly intake of some cruciferous vegetables in the UK, USA, Canada and Japan (g/person)*

| Vegetables | UK (1980) | USA (1978) | Canada (1978) | Japan (1975) |
|---|---|---|---|---|
| Cabbage | 123·2 | 77·0 | 34·2 | 136·5 |
| Brussels sprouts | 67·9 | 2·1 | 5·2 | — |
| Broccoli | — | 23·1 | 14·1 | — |
| Cauliflower | 71·4 | 14·0 | 9·4 | — |
| Chinese cabbage | — | — | 0·4 | 159·6 |
| Turnip/Swede | 38·5 | — | 24·6 | — |
| Mustard | — | — | 8·3 | — |
| Radish | — | — | 7·2 | 232·4 |
| Coleslaw | — | — | 14·3 | — |
| Sauerkraut | — | 11·2 | 6·7 | — |
| Salt fermented vegetables | — | — | — | 260·4 |

References are: Benns *et al.* (1978), Fenwick *et al.* (1982), Sones *et al.* (1984*a*).

Table 5. *Average weekly intake of glucosinolates from fresh vegetables in the UK and Canada*

| | UK (1980) | | Canada (1978) | |
|---|---|---|---|---|
| | Glucosinolate content (mg/100 g fresh weight) | Glucosinolate intake (mg/person) | Glucosinolate content (mg/100 g fresh weight) | Glucosinolate intake (mg/person) |
| Cabbage | 108·9 | 135·8 | 23·7 | 11·5 (Including coleslaw) |
| Brussels sprouts | 226·2 | 120·4 | 122·4 | 6·4 |
| Broccoli | — | — | 29·3 | 4·2 |
| Cauliflower | 62·0 | 44·8 | 32·09 | 3·0 |
| Turnip/Swede | 56·0 | 21·7 | 122·6 | 30·2 |
| Radish | — | — | 11·8 | 0·9 |
| | | 322·7 | | 56·2 |

References are: Mullin & Sahasrabudhe (1978), Sones *et al.* (1984*a*).

Living standards may also account for variations in cruciferous vegetable consumption; as the income increases, there is an increase in total fresh green vegetable consumption (Sones *et al.* 1984*a*) and, among them, mild flavoured vegetables such as cauliflower or calabrese are preferred to cabbage or kale (Crisp, 1976 cited in Lewis & Fenwick, 1987). Sones *et al.* (1984*a*) and Mullin & Sahasrabudhe (1978) have estimated, from the British and Canadian consumption data reported in Table 4, the average intake of GSL in British and Canadian populations respectively. Assuming that the vegetables were eaten raw, the mean daily intakes were calculated to be 8·0 and 46·1 mg respectively in Canada and the UK (Table 5). Figures for individual GSL or GSL derivatives have occasionally been reported by some authors; the intake of progoitrin in the average UK diet is approximately 7 mg/day (Fenwick *et al.* 1983) and an average level of 28 $\mu$mol of glucoiberin is reported to be ingested daily by US citizens (Kore *et al.* 1993). Although this kind of information is very useful to draw a picture of levels of GSL ingested by humans, it must be treated with extreme caution since the final GSL and GSL derivative content of a dietary cruciferous

vegetable depends on a tremendous number of factors. The researchers were of course aware of this uncertainty; indeed Sones *et al.* (1984*a*) have estimated that the amount of GSL ingested by certain individuals could exceed 300 mg/day.

On the whole, the findings reported here on GSL content of cruciferous vegetables and subsequent GSL consumption demonstrate that investigations about the biological effects of GSL should include measurement of, at the very least, the total GSL content and, ideally, of the content of individual GSL and hydrolysis products of the cruciferous vegetable included in the experimental diets. In addition, detailed information on how the cruciferous material and food are processed should also be provided; this should help nutritionists and toxicologists to obtain more valuable information from studies in which the experimental diets are inevitably different. Furthermore, if estimated figures for GSL consumption are helpful tools for the design of experimental diets, one should keep in mind that tremendous quantitative and qualitative variations occur in GSL consumption by humans.

## TOXICITY OF GLUCOSINOLATES AND GLUCOSINOLATE DERIVATIVES

GSL derivatives are now known to be the toxic principles of cruciferous vegetables, and their toxic effects are well documented, especially in the case of rapeseed meal. Summarizing the vast literature published on this issue would be far too long; we have therefore stressed only the most striking points.

Experimentally, the general phenomenon observed is impaired performance of animals consuming GSL-rich feeds. The gross toxic effects can be described as reduced feed intake, growth depression, enlargement of target organs (liver, kidneys, thyroid gland) and reproductive disorders such as embryo mortality in mammals and decreased egg production in birds. The intensity of these effects varies with the animal species and, of course, the amount of GSL in their food (Bourdon *et al.* 1981; Butler *et al.* 1982; Bell, 1984; Vermorel *et al.* 1987; Etienne & Dourmad, 1987). In humans, reduced iodine uptake by the thyroid gland was reported after daily ingestion of 500 g cabbage for 2 weeks (Langer *et al.* 1971) or after a single meal of 300 to 500 g swede or turnip (Greer & Astwood, 1948). However, a more recent study by McMillan *et al.* (1986) did not lead to hypothyroidism in human volunteers consuming 150 g Brussels sprouts daily for 4 weeks. Nevertheless these contradictory findings are not too puzzling, since the thyroid function indices that were examined and the cruciferous vegetables and their GSL that were ingested were not the same.

Several attempts have been made to ascertain precisely which GSL or GSL derivatives are responsible for the different components of GSL toxicity. Addition of pure sinigrin or gluconapin to the diet led to liver hypertrophy in rats. Progoitrin seems to have a greater toxic potential; it has been shown to induce enlargement of the liver, kidneys and thyroid in rats (Bille *et al.* 1983; Vermorel *et al.* 1986). Goitrin (5-vinyloxazolidine-2-thione), one of the major derivatives of progoitrin, has been the most extensively studied GSL derivative, as far as toxicity is concerned. This goitrogen, very potent even at low doses (Krusius & Peltola, 1966; Langer & Michajlovskij, 1969; Akiba & Matsumoto, 1976), can induce decreased uptake of iodine by the thyroid gland in humans (Astwood *et al.* 1949) and in rats, modify the triiodothyronine:thyroxine ratio and alter the histological pattern of the thyroid in rats (Lo & Hill, 1971; Bell *et al.* 1972; Lo & Bell, 1972). It seems that goitrin interferes with organic iodination of thyroxine precursors in the gland, thus leading to compensatory goitre (Akiba & Matsumoto, 1976; Elfving, 1980). Isothiocyanates and thiocyanates were held responsible for similar thyroid disorders (Langer, 1964 cited in

Duncan & Milne, 1989; Langer & Štolc, 1965). The former prevent the iodination of tyrosine, as does goitrin, whereas the latter are known competitively to inhibit iodine uptake by thyroid cells (Langer & Greer, 1968; Muztar *et al.* 1979). Sinigrin and glucoiberin isothiocyanate derivatives were also shown to induce embryo death in the rat but the mechanism is still unknown (Nishie & Daxenbichler, 1980). Preferential target organs of the nitrile derivatives seem to be the liver and kidneys (VanEtten *et al.* 1969; Srivastava *et al.* 1975). The mechanism that underlies their toxicity seems to be their ability to interact with reduced glutathione, thus leading to substantial alterations in tissue glutathione levels as observed by Szabo *et al.* (1977) in the liver, kidneys, adrenals and lungs of rats after chronic ingestion or a single injection of acrylonitrile. The toxic effect of nitriles manifests itself as hypertrophy of the target organs, disruption of the normal lobular structure of the liver and irregular proliferation of the bile duct (VanEtten *et al.* 1969). As far as kidneys are concerned, enlarged nuclei of the epithelial cells lining the convoluted tubules have been observed (VanEtten *et al.* 1969). Gould *et al.* (1985) observed rapid production of kidney lesions, along with elevated plasma levels of nitrogen, urea and creatinine, which could suggest functional alterations of the kidneys.

Investigating the nature and the underlying mechanisms of toxic effects induced by GSL derivatives released by plant myrosinase gives very valuable information. However, it does not take into account the ability of the intestinal microflora to break down intact GSL or their derivatives into metabolites of which the nature and specific toxic potential are so far largely unknown. Experiments with gnotobiotic animals have proved to be an invaluable tool for addressing this topic. Those performed in our laboratory suggest that the different toxic patterns usually observed in different animal species are more likely to be due to differences in the autochthonous digestive microflora than to intrinsic host sensitivity toward GSL. Indeed, when given a diet based on rapeseed meal, conventional rats exhibit GSL-linked symptoms different from those of gnotobiotic rats harbouring either chicken or human microflora (Nugon-Baudon *et al.* 1988; Rabot *et al.* 1993*a*). The inoculation of germ free rats with single strains of fowl or human origin provided further information about the role of intestinal microflora in the production of toxic GSL derivatives. The toxic effects, observed in rats associated with a whole human microflora, namely reduced feed intake and weight gain, enlargement of the liver and thyroid and a decrease in both thyroxine (T4) and triiodothyronine (T3) plasma levels, could be reproduced in gnotobiotic rats harbouring a single human strain of *Bacteroides vulgatus* (Rabot *et al.* 1993*a*). Eventually, such simplified gnotobiotic models enabled our group to split the toxicity observed with complex intestinal microflora into different patterns (Table 6). A *Lactobacillus* strain isolated from a chicken crop was shown to induce goitre in gnotobiotic rats given a diet based on rapeseed meal (Nugon-Baudon *et al.* 1990*b*) whereas human strains of *Clostridium butyricum* and *Escherichia coli*, each isolated from healthy individuals, were responsible for liver hypertrophy and goitre associated with reduced T4 and T3 plasma levels respectively (Rabot *et al.* 1991, 1993a).

These findings reinforce the idea that, should the plant myrosinase in the diet be totally inactivated, the toxicity of GSL would depend strictly on the equilibrium between bacterial species possessing specific myrosinase-like activities. There exists an overall similarity in the nature of toxic effects observed in conventional rats given either pure GSL derivatives produced by plant myrosinase or a GSL-rich but myrosinase free diet. Nevertheless, one cannot exclude the possibility that extra metabolites, toxic or non-toxic, may be produced either from intact GSL or from previously released derivatives. This would of course enhance the difficulty that one encounters when trying to infer the potential toxicity of a diet containing cruciferous vegetables from a knowledge of its GSL and GSL derivative content.

Table 6. *Effects of a diet with rapeseed meal on weight gain, organ weight and thyroid hormones in gnotobiotic rats according to their bacterial status*

(Results are expressed as % of the mean values obtained with counterpart rats given a diet with soyabean meal)

| Bacterial strain... | *Lactobacillus* (a) (LEM 220 strain) | *Bacteroides vulgatus* (b) (BV8H1 strain) | *Clostridium butyricum* (b) (CB1002 strain) | *Escherichia coli* (b) (EM0 strain) |
|---|---|---|---|---|
| Reference... | Nugon-Baudon *et al.* (1990*b*) | Rabot *et al.* (1993*a*) | Rabot *et al.* (1990) | Rabot *et al.* (1993*a*) |
| Duration of the trial (weeks)... | 5 | 7 | 7 | 7 |
| No. of animals... | 7 | 6 | 6 | 6 |
| Cumulative weight gain | 109 | 28*** | 105 | 90 |
| Liver | 106 | 114*** | 115** | 101 |
| Kidneys | 99 | 113 | 108** | 95 |
| Thyroid | 148*** | 672*** | 111 | 302*** |
| Tetraiodothyronine | 112 | 57** | 95 | 56*** |
| Triiodothyronine | ND | 71 | ND | 71** |

Mean values were significantly different from those for counterpart animals given a soyabean meal diet: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

(a) Isolated from a chicken crop.

(b) Isolated from the faecal flora of adult healthy humans.

ND: not determined.

## GLUCOSINOLATES AND GLUCOSINOLATE DERIVATIVES: NEW CANDIDATES FOR PROTECTION AGAINST CHEMICAL CARCINOGENESIS

### EPIDEMIOLOGICAL DATA: CRUCIFEROUS VEGETABLES AND CANCER INCIDENCE IN HUMAN POPULATIONS

Toxic effects of GSL and their derivatives in humans have seldom been described; in animals they are now less dramatic since new varieties of rape containing very low amounts of GSL have been bred. Nevertheless an ever increasing number of publications suggest a new potential of GSL-containing vegetables, namely that they may be serious candidates for protection against chemically induced cancer.

Different epidemiological studies (Graham *et al.* 1972, 1978; Haenzsel *et al.* 1980) seem to support the hypothesis that the consumption of cruciferous vegetables is associated with a lower risk of tumour formation in the human digestive tract (stomach, colon, rectum). Such observations led the (American) Committee on Diet, Nutrition and Cancer (1982) to suggest that the consumption of cruciferous vegetables "was associated with a reduction in the incidence of cancer at several sites in humans".

### EXPERIMENTAL DATA: CRUCIFEROUS VEGETABLES, GLUCOSINOLATES AND CHEMICAL CARCINOGENS IN ANIMAL MODELS

The remarkable work carried out by Stoewsand and his team was a determining step in the experimental demonstration of the potentially beneficial effects of GSL consumption on chemically induced cancers. An initial experiment by these authors showed that giving rats a diet with 20% freeze-dried cauliflower reduced the toxic effects of aflatoxin B1 given

orally (Stoewsand *et al.* 1978). This was subsequently supported by other studies by Boyd *et al.* (1982) using a diet with 25% freeze-dried cabbage, and by Salbe & Bjeldanes (1989) using a diet with 25% chopped and freeze-dried Brussels sprouts. The latter authors showed that aflatoxin B1 binding to hepatic DNA was much decreased when rats had been given Brussels sprouts for 2 weeks prior to the intraperitoneal or intragastric administration of the toxin. Female rats were also significantly protected against the carcinogenic properties of 7,12-dimethylbenz(*a*)anthracene administered by oral intubation when they received a feed containing 20% freeze-dried Brussels sprouts during the initiation period of carcinogenesis; in this 2-week experiment, the incidence of mammary tumours induced by 7,12-dimethylbenz(*a*)anthracene dropped from 77% in the control animals to 13% in the animals consuming the cruciferous vegetable (Stoewsand *et al.* 1988). Other studies have highlighted the protective effect of GSL-rich diets against chemically induced tumours (Wattenberg & Loub, 1978; Wattenberg *et al.* 1986).

However, these results showing the anticarcinogenic properties of cruciferous vegetables are counterbalanced by another series of experiments. Diets with 10% dried cabbage, for instance, have been shown to increase the incidence of pancreatic ductular carcinomas induced by *N*-nitroso-bis(2-oxopropyl)amine (Birt *et al.* 1987) in mice. A study carried out by Srisangnam *et al.* (1980*a*) is even more equivocal; the authors concluded that diets containing 10–20% sliced dehydrated cabbage enhanced the tumorigenicity of 1,2-dimethylhydrazine in mice whereas 40% cabbage in the feed has a protective effect. Although these results are very interesting, it is important to emphasize that in these cases 1,2-dimethylhydrazine and *N*-nitroso-bis(2-oxopropyl)amine were injected subcutaneously and that the greatest incidence of tumours was obtained with a high fat diet (22%; Birt *et al.* 1987).

The discrepancies observed between the findings can probably be explained partly by the tremendous variations in experimental design with respect to variables such as animal species, strain, sex, age, etc., the nature of the cruciferous vegetable and/or of the carcinogenic agent, the route of administration and/or the duration of the experiment. On the whole, these findings, albeit inconsistent, give definite evidence of the influence of cruciferous vegetables on chemical carcinogenesis.

In elucidating the anticarcinogenic properties of cruciferous vegetables, much of the work has focused on the effects of purified indolylGSL and derivatives. The main compound tested in these studies has been glucobrassicin and, more precisely, the derivatives obtained *via* its hydrolysis by plant myrosinase. When orally intubated into female rats before the administration of 7,12-dimethylbenz(*a*)anthracene, I3C (0·10 mmol/rat) and 3,3′-diindolylmethane (0·05 mmol/rat), but not IAN (0·10 mmol/rat), significantly reduced the incidence of mammary tumours (Wattenberg & Loub, 1978). Furthermore, mice given orally a 12 mg dose of the parent compound, glucobrassicin, a few days or even a few hours (4 h) before oral administration of benzo(*a*)pyrene (BaP), developed fewer forestomach and lung tumours (Wattenberg *et al.* 1986). In rats, I3C (1 g/kg diet) was shown to inhibit the hepatocarcinogenesis induced by diethylnitrosamine (40 mg/l drinking water) when it was administered concurrently with the carcinogen (Tanaka *et al.* 1990). Shertzer (1983, 1984) studied the change in binding to DNA of BaP or *N*-nitrosodimethylamine (NDMA) metabolites after mice were given I3C by gavage (163 mg/kg body weight); in both cases, there was evidence of a dramatic decrease in covalent binding. In contrast I3C proved unable to decrease the binding of aflatoxin B1 to hepatic DNA, whether it was administered *via* the intraperitoneal route or by gavage (Salbe & Bjeldanes, 1989). Pence *et al.* (1986) even demonstrated that I3C incorporated into the diet at a level of 1 g/kg dry matter enhanced 1,2-dimethylhydrazine induced tumorigenicity in rats; in this experiment, 1,2-dimethylhydrazine (10 mg/kg body weight) was injected

intraperitoneally weekly for 16 weeks and the enhancing effect of I3C was significantly increased when the animals were given a high fat (20%) diet.

Apart from sinigrin which was shown to exhibit a protective effect similar to that of I3C against diethylnitrosamine induced hepatocarcinogenesis (Tanaka *et al.* 1990), other GSL have received little or even no attention, so that a great deal of uncertainty remains about the extent to which GSL can impede the carcinogenic process. Nevertheless, as was observed with diets based on cruciferous vegetables, the effects of glucobrassicin or of some of its derivatives are not always protective. One of the most likely explanations for these inconsistent results, i.e. enhancement versus reduction of the incidence of cancers in experimental animal models, is that GSL and/or GSL derivatives may modify the endogenous system of xenobiotic metabolizing enzymes (XME).

## EXPERIMENTAL DATA: CRUCIFEROUS VEGETABLES, GLUCOSINOLATES AND XENOBIOTIC METABOLIZING ENZYMES

### *The xenobiotic metabolizing enzymes*

We do not explain in detail how the XME system, which is very complex, works, since excellent reviews have been published (Burke & Orrenius, 1979; Kato, 1979; Caldwell, 1980). We give only a few examples which indicate the ways in which GSL can interfere with this biotransformation–detoxification system and help our understanding, at least in part, of their deleterious or protective effects.

The XME system is ubiquitous (skin, intestine, lungs, kidneys) with some exceptions, but is present mainly in the liver (Beaune, 1982, 1986). The reactions catalysed by the XME confer hydrophilic properties on endogenous compounds or molecules entering the organism that would otherwise be hard to eliminate due to their rather hydrophobic nature.

This system is usually described as having two phases, although a compound may be metabolized by either one or both phases (Jakoby, 1980). Phase one is represented by different enzymes such as flavin-containing monooxygenase, alcohol and aldehyde dehydrogenases, etc. The most widely studied enzymes belonging to phase I are undoubtedly the cytochrome P450 family (*EC* 1.14.14.1), probably because they metabolize a tremendous number of substances (Jakoby, 1980). We do not go into details of the biochemistry of the reactions catalysed by P450; schematically, these microsomal monooxygenases incorporate one atom of molecular oxygen into an organic substrate while using reducing equivalents ($NADPH/H^+$) to reduce the remaining oxygen atom to water. Since the discovery of cytochrome P450 by G. R. Williams in B. Chance's laboratory in 1955 (Conney, 1982), it has become obvious that it plays a key role in the metabolism of many xenobiotic or endogenous substances. So far approximately twenty P450 isoenzymes in the liver of the rat have been described (Nebert *et al.* 1989). As far as human P450 are concerned, results are of course less straightforward, due to the wide differences that may exist between individuals (genetic background, xenobiotic exposure, dietary habits, etc.; Wrighton *et al.* 1986; Guengerich, 1989; Sesardic *et al.* 1990). Individual forms of P450 may exhibit different degrees of specificity toward multiple substrates, i.e. high $K_m$ activities toward some substrates and low $K_m$ activities toward others.

The reactive products released by phase I can be further metabolized by phase II enzymes. Phase II catalyses the conjugation of phase I intermediates with endogenous ligands such as amino acids, glucuronic acid, sulphate or glutathione. As for P450, phase II is represented by large families of isoenzymes with overlapping substrate specificities (Habig *et al.* 1974; Jakoby, 1978; Wishart, 1978; Bock *et al.* 1979). UDPglucuronosyltransferases (GT, *EC* 2.4.1.17) are microsomal enzymes that catalyse conjugation with

UDPglucuronic acid (Bock *et al.* 1987; Burchell *et al.* 1987). It seems that glucuronidation is the most important form of conjugation. Three of the isoenzymes identified so far in the rat are involved in the glucuronidation of endogenous substrates such as bilirubin and steroid hormones. Hepatic glucuronides are usually excreted *via* the bile. Most of the compounds that can be glucuronidated can also be sulphated by sulphotransferases (*EC* 2.8.2.1 etc.). These enzymes are located in the cytosol and catalyse the formation of sulphate monoesters with 3′-phosphoadenosine-5′-phosphosulphate. The result of the competition for a substrate between sulphotransferases and GT is usually in favour of the former, at least when the substrate concentration is low. With the exception of one microsomal form, glutathione *S*-transferases (GST, *EC* 2.5.1.18) are cytosolic proteins which conjugate glutathione on the sulphur atom of cysteine to various electrophiles (Mannervik, 1985; Pickett & Lu, 1989; Coles & Ketterer, 1990). GST also play a key role in the transport of hormones to the cell nucleus. Epoxide hydrolases (EH, *EC* 3.3.2.3) are found in both the cytosol and the endoplasmic reticulum. Their action is important since they degrade reactive epoxides by the addition of water, thus generally leading to the less reactive diols. However EH can sometimes contribute to the genesis of potent carcinogens as seen with the transformation of BaP: the EH mediated 7,8-dihydrodiol metabolite is less reactive than the parent molecule but cytochrome P450 can convert it into an extremely reactive epoxide responsible for the well-known mutagenic and carcinogenic properties of BaP. As with all other enzymes so far described, EH is also involved in the biotransformation of endogenous intermediates such as oestrogen and androgen epoxides (Timms *et al.* 1987).

Depending on their molecular weight, structure and polarity, conjugated metabolites are eliminated *via* urine or bile. Before urinary excretion, glutathione conjugates are further metabolized into mercapturic acids. Metabolites excreted *via* the biliary route may be partly hydrolysed by intestinal microflora and reabsorbed. This last transformation constitutes the first step of an enterohepatic cycle (Rowland, 1988).

Although XME is usually considered a detoxification system, such is not always the case. A lot of examples are known where it enhances or generates toxicity (carcinogenicity). On the whole it seems that the role of P450 in the toxification *v.* detoxification balance is far more ambiguous than that of phase II transferases. It is usually accepted, with some exceptions such as morphine-6-glucuronide (Caldwell, 1979), that an increase in the specific activities of transferases enhances detoxification. The XME system is very versatile and many factors may modulate its capacity. Apart from genetic characteristics (species, gender, individual), inducers may specifically enhance some of its activities, thus orienting its detoxification or toxification potential (Conney, 1982; Guengerich *et al.* 1982; Ullrich & Bock, 1984). Consequently, the induction of an isoform of P450 by a xenobiotic can have grave consequences for the fate of another xenobiotic, particularly if the latter is activated into a reactive toxic or carcinogenic metabolite by the isoform.

*The effects of cruciferous vegetables on the XME system*

A lot of work has been done since epidemiological and experimental findings first supported the idea of a protective role of GSL-rich diets against cancer. Most researchers have tried to elucidate the mechanism by which GSL and/or their derivatives could alter the XME system, both in the liver and the intestine.

Historically, the first work on that topic was performed by Wattenberg (1971) who showed that BaP hydroxylation in the rat intestine was very much enhanced when the animals were given a cabbage based diet. This study was then extended to other cruciferous vegetables and other activities of the phase I XME. McDanell *et al.* (1989) described the enhancement of ethoxyresorufin deethylation activity in the small intestine (5-fold), in the

colon (4-fold) and in the liver (2·5-fold) of rats given a diet with 25% freeze-dried Brussels sprouts for 6 d. Similarly, feeding rats for 2 weeks on a diet containing 25% chopped and freeze-dried Brussels sprouts led to the induction (2-fold) of intestinal aryl hydrocarbon hydroxylase and ethoxycoumarin *O*-deethylase activities (Salbe & Bjeldanes, 1989). However no induction was seen in the liver, as already reported by Hendrich & Bjeldanes (1983) in mice fed on diets containing 20% chopped and freeze-dried cabbage or Brussels sprouts. A single meal of a GSL-containing food (25% dried cabbage) is not enough to modify the ethoxyresorufin deethylation activity in the liver and colon but it succeeds in inducing a temporary enhancement of this activity in the small intestine, the peak occurring 4–6 h post ingestion (McDanell *et al.* 1989). In our laboratory, monoclonal antibodies were used to investigate the influence of a diet with 39% rapeseed meal on the isoenzyme pattern of P450 in the liver of male rats. After 4 weeks, an overall reduction in the total P450 (−25%) occurred resulting from a 66% decrease of the 2C11 (male constitutive) form whereas the 1A1/1A2 (polycyclic hydrocarbon inducible) form was enhanced by 61%; the 2B1/B2 (phenobarbital inducible), 2E (ethanol inducible) and 3A (steroid inducible) forms also measured were not significantly modified (Nugon-Baudon *et al.* 1991).

Concerning phase II enzymes, giving rats for 10 d a diet containing 25% freeze-dried Brussels sprouts was shown to induce hepatic and intestinal GST and intestinal EH (Bradfield & Bjeldanes, 1984). Such phenomena were also observed by Aspry & Bjeldanes (1983) using diets containing 10–25% chopped freeze-dried broccoli. We have reproduced the induction of hepatic GST (2·5-fold) in rats given a diet with 39% rapeseed meal for 4 weeks and extended the investigations to hepatic GT; the activity of this last conjugative enzyme was dramatically enhanced (4-fold; Nugon-Baudon *et al.* 1990*a*). As far as hepatic EH is concerned, a slight stimulation (1·4-fold) was observed in mice fed for 10 d on a diet containing 20% chopped and freeze-dried Brussels sprouts but it did not occur when Brussels sprouts were replaced with cabbage (Hendrich & Bjeldanes, 1983).

The effects of various cruciferous vegetables on the phase I system seem to vary. Discrepancies between the experimental designs could be held responsible; indeed, Miller & Stoewsand (1983) have clearly shown that the phase I system of different strains of rats responded in different ways to a cabbage-containing diet. In contrast, results obtained on phase II are less divergent and there is now strong evidence of an overall induction of transferases in the intestine as well as the liver, whatever the experimental design.

It is now well known that the content and pattern of intact GSL and GSL derivatives are very much affected by the processing operations undergone by cruciferous vegetables before consumption. As cooking is one of the most usual treatments the question is, do cooked cruciferous vegetables modify the intestinal and/or hepatic XME, and if so how? Very few studies have been published on this issue. Recently, Wortelboer *et al.* (1992) have addressed the topic, using Brussels sprouts cooked for 20 min in unsalted water; consequently, the total GSL concentration dropped from 7·3 to 4·9 mmol/kg dry matter. Rats were given semi-synthetic diets containing either 0, 2·5, 5 or 20% cooked Brussels sprouts on a dry matter basis. Animals of each dietary group were killed after 2, 7, 14 or 28 d in order to assess the effects of the different levels of Brussels sprouts on hepatic and intestinal phase I and phase II enzymes. GST activity was induced throughout the experiment, in the intestine only by the 20% diet, and in the liver by diets containing at least 5% Brussels sprouts. From 2 d treatment onwards, the 20% diet also induced hepatic NAD(P)H quinone reductase (*EC* 1.6.99.2) and GT1 activities but it decreased hepatic GT2 activity. As far as P450 isoenzymes are concerned, polycyclic hydrocarbon inducible forms, i.e. 1A2 in the liver and 2B1/B2 in the small intestine, were induced in a dose related manner by all diets containing Brussels sprouts throughout the experiment. Apart from the immunochemical detection of apoproteins, the authors have used marker substrates to try

to correlate Western-blot results and enzyme activities. Some were possible: enhanced ethoxyresorufin deethylation activity in the liver is correlated with the induction of 1A2, and increased 16$\alpha$- and 16$\beta$-hydroxylation of testosterone by intestinal microsomes is correlated with the induction of the intestinal 2B isoenzyme. These results are quite important, since they show that cruciferous vegetables processed in the way that they usually are in a human diet may alter very significantly the XME system.

*The effects of glucosinolates and glucosinolate derivatives on the XME system*

Consistent with the work performed on the anticarcinogenic properties of pure GSL and GSL derivatives, most studies investigating the GSL linked alterations of the XME have strongly focused on indolylGSL and their enzymic derivatives.

Of four pure GSL, sinigrin, progoitrin, glucotropaeolin and glucobrassicin, only the last compound has been shown to induce phase I enzymes significantly, at least in the rat small intestine (McDanell *et al.* 1989). Loub *et al.* (1975) repeated the original work of Wattenberg (1971) on BaP hydroxylation, using pure I3C, 3,3′-diindolylmethane, IAN and ascorbigen as potential XME inducers. Given to rats by gavage a few hours before they were killed, these glucobrassicin derivatives induced BaP hydroxylation in the liver and the small intestine. I3C was tremendously active: a single 0·1 mmol dose induced 56- and 31-fold enhancements of BaP hydroxylation in the liver and small intestine respectively. Related studies, in which three glucobrassicin derivatives were given to rats twice daily for 3 d, corroborate this result (Pantuck *et al.* 1976); 3,3′-diindolylmethane (175 mg/kg body weight), IAN (95 mg/kg) and, to an even more important extent, I3C (100 mg/kg) each increased the intestinal metabolism of phenacetin, 7-ethoxycoumarin, hexobarbitone and BaP. I3C can modify the metabolism of other chemicals as well. In rainbow trout, dietary I3C (2 g/kg diet on a dry matter basis) is involved in substantial changes in the distribution, metabolism and elimination of aflatoxin B1, leading to significantly reduced hepatic DNA damage (Goeger *et al.* 1986). In liver microsomes prepared from rats fed for 2 weeks on an I3C-containing diet (30 mmol/kg on a dry matter basis) $\alpha$-hydroxylation of NDMA and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which are environmentally prevalent nitrosamines, is enhanced; in this case, the inducing effect of I3C is particularly harmful since it enhances the release of reactive intermediates binding to DNA (Chung *et al.* 1985). Bradfield & Bjeldanes (1984) reported that a dose of I3C as low as 50 mg/kg synthetic diet, given to rats for 10 d, led to a 6-fold increase of BaP hydroxylation activity in the small intestine. Gradually increasing the dose up to 500 mg/kg led to a positively correlated level of induction. The same effect was seen with intestinal ethoxycoumarin *O*-deethylase. However no effect on the hepatic counterparts of these activities could be seen, whatever the diet and the I3C concentration, though a slight increase in total P450 concentration occurred when animals were given the 500 mg/kg I3C diet. Shertzer (1982) also found contrasting results when he administered comparable doses of IAN or I3C to mice, rats and rabbits, orally or *via* the intraperitoneal route, daily for 10 d: IAN had no effect on either hepatic cytochrome P450 or BaP hydroxylation activity in any of the three animal species; with I3C, a 2-fold induction of hepatic P450 and BaP hydroxylation could be seen in the liver of mice and rats but not rabbits. On the whole, the induction level was much weaker than those reported elsewhere, especially considering that the administration of the GSL derivatives lasted a rather long time. The controversy increased with the findings of Babish & Stoewsand (1978); using rats given dietary levels of I3C ranging from 50 to 7500 mg/kg diet for 3 weeks, these authors observed a significant induction of intestinal BaP hydroxylation activity only at a dose that would correspond to a daily intake of 1·5 g/kg body weight, which is totally unrealistic for a human diet! Therefore the authors concluded that I3C is not the major inducer of phase I activities.

Once again, it is regrettable that so few studies have been conducted on other GSL or GSL derivatives. Nevertheless, among them, goitrin has received particular attention. According to Chang & Bjeldanes (1985), goitrin given to rats does not alter the ethoxycoumarin *O*-deethylase activity, either in the liver or in the small intestine, even at the lowest dose tested (40 mg/kg diet for 14 d). Recently Ozierenski *et al.* (1993) concluded that dietary goitrin is able to modify phase I activities in the rat liver, though in a contrasting way; whereas no significant modification of the overall P450 concentration occurred, aminopyrine *N*-demethylation was reduced and aniline *p*-hydroxylation was enhanced in a dose dependent manner. Ozierenski *et al.* (1993) have extended their investigations to a series of isothiocyanate derivatives and to 1-cyano-3-butene, the nitrile derived from gluconapin; on the whole, the overall concentration of P450 is significantly reduced and is accompanied by a dose related decrease of several P450 dependent activities such as aminopyrine *N*-demethylation, aniline *p*-hydroxylation and *p*-nitroanisole *O*-demethylation. Such results support our own findings concerning the heterogeneous alterations of the isoenzyme profile of P450 in the liver induced by rapeseed meal (Nugon-Baudon *et al.* 1991). *In vivo* consequences of the alterations of P450 activities by isothiocyanates have been investigated by Chung *et al.* (1985). These authors showed that isothiocyanates such as allyl-, benzyl- and phenylethylisothiocyanate, derived from sinigrin, glucotropaeolin and gluconasturtiin respectively, were good inhibitors of NDMA and NNK $\alpha$-hydroxylation in liver microsomes prepared from rats fed for 2 weeks on a diet containing one of these compounds (3 mmol/kg dry matter); similar treatment with sinigrin also caused a significant decrease in the $\alpha$-hydroxylation of these nitrosamines. In view of their promising inhibitory activities, the effects of dietary phenylethylisothiocyanate and sinigrin on the *in vivo* methylation of DNA by NDMA (25 mg/kg body weight by intraperitoneal injection) and NNK (85 mg/kg body weight by intravenous injection) were evaluated. The results were parallel to those obtained in the *in vitro* assays, suggesting that these compounds might be potent inhibitors of NDMA and NNK carcinogenesis.

Compared with phase I activities, the influence of GSL and GSL derivatives on phase II XME has been less fully investigated. Sparnins *et al.* (1982) showed that a semi-purified diet containing 6 g/kg I3C induced intestinal and hepatic GST (3-fold) after a 10 d trial in mice. A comparable result was reported later on hepatic EH (Cha *et al.* 1985). In both studies, the levels of I3C were very high and not to be found in a human diet. Using a diet containing 0·5 g/kg I3C, Wortelboer (1991) found a slight induction of liver and intestinal GST after 2 d and an induction of GT after 7 d in the rat. Nevertheless, other authors have published results that tend to show that no induction of intestinal or hepatic GST or EH activities by I3C is possible at normal dietary levels (Bradfield & Bjeldanes, 1984; Salbe & Bjeldanes, 1989). Although a diet with Brussels sprouts given to rats for 10 d induces both GST and EH in the liver as well as in the small intestine, synthetic diets containing 50–500 mg/kg I3C do not alter these activities at all (Bradfield & Bjeldanes, 1984).

There is now firm evidence that glucobrassicin and its derivatives cannot exclusively account for the phase II alterations observed when feeding crucifer-containing diets, far from it. This point has prompted several groups to look for effects of other GSL and GSL derivatives. One of the inducing molecules for phase II enzymes was identified as goitrin (Chang & Bjeldanes, 1985); when given to rats (40 mg/kg diet) for 14 d, this progoitrin derivative was able to increase significantly hepatic GST and EH activities. Ozierenski *et al.* (1993) addressed the same point, comparing the effects of goitrin and the gluconapin derivatives, 1-cyano-3-butene and butenyl isothiocyanate, and various isothiocyanates on GST in rat liver. All compounds tested, other than 1-cyano-3-butene, caused an increase in GST activity. In another very recent study, Zhang *et al.* (1992) applied a glucoiberin derivative, 1-isothiocyanato-(3*R*)-(methylsulphinyl) propane (IMSP), and a glucoraphanin

derivative, 1-isothiocyanato-(4*R*)-(methylsulphinyl) butane, to a Hepa 1c1c7 murine hepatoma cell culture and found that both these derivatives were potent inducers of GST and NAD(P)H quinone reductase. These results were of extreme importance and deserved to be confirmed and qualified *in vivo*, which was done by Kore *et al.* in 1993. IMSP doses of 1, 10 and 100 $\mu$mol/kg body weight were given by gavage to rats once daily for 7 d; the lowest dose was, according to the authors, comparable to what an average western diet would contain. No alterations, whatever the dose, could be seen in hepatic levels of cytochrome P450, ethoxycoumarin *O*-deethylase or aminopyrine *N*-demethylase activities or in hepatic quinone reductase, GST and GT. However an important induction of intestinal quinone reductase (8-fold) and a moderate induction of intestinal GST (2-fold) occurred, but only at the highest dose of IMSP. Thus it was concluded that the IMSP content occurring in an average human diet may have no significant influence on either phase I or phase II enzymes.

All these findings definitely show that the XME alterations mediated by cruciferous vegetables involve many kinds of GSL derivatives. The numerous dose related studies reported here highlight the fact that one must be extremely cautious in extrapolating alterations observed under experimental conditions to real nutritional conditions; it seems that some molecules, albeit undeniably active towards XME, are eventually not relevant from a nutritional point of view and should rather be considered as candidates for pharmacological investigation.

## CONCLUSIONS AND PENDING TOPICS

One of the major points which remains to be addressed is of course how far it is possible to extrapolate to humans results established in laboratory rodents.

Only a few studies have been performed so far in humans, for obvious reasons. Nevertheless the pharmacological fates of some drugs which are known to be metabolized by the XME system have been examined by Pantuck and coworkers in volunteers consuming cruciferous vegetables. The metabolism of phenacetin and antipyrine and the glucuronidation of paracetamol are enhanced by the consumption of Brussels sprouts, cabbage and other cruciferous-containing diets (Pantuck *et al.* 1979, 1984). In a review on indolylGSL, McDanell *et al.* (1988) support the idea that GSL derivatives are likely to be as active on the XME system of humans as they are in laboratory animals.

Among the most striking points, when looking at the findings reported in the present review, are the conflicting results which appear to arise from the diversity of amounts of cruciferous vegetables and GSL or GSL derivatives incorporated in the rodent diets. Furthermore, where cruciferous vegetables have been used, the reader has sometimes been poorly informed on the GSL content resulting from the process undergone by the vegetable before its incorporation into the diet. Since cruciferous vegetables are usually eaten after treatments such as mashing, fermenting, cooking, etc., reports on the effects of such treatments on the GSL related XME alterations would be a crucial matter to develop in order to extend data provided by the original works of McDanell *et al.* (1987), Wortelboer (1991) and Wortelboer *et al.* (1992).

All the studies reported here deal with the impact of GSL on environmental procarcinogens and/or carcinogens biotransformed via the XME system. Very few studies have investigated the extent to which GSL may alter steroid metabolism, though this point could be important for hormone dependent cancers in the human. Michnovicz & Bradlow (1991) have shown that in twelve healthy men and women ingestion of 6–7 mg/day of I3C for 7 d increased the 2-hydroxylation of oestradiol by about 50%, thus enhancing the urinary excretion of 2-hydroxyoestrone relative to the excretion of oestriol. In a

Table 7. *Effects of a diet with rapeseed meal on three hepatic xenobiotic metabolizing enzymes in germ free and conventional rats*

(Results are expressed as % of the mean values obtained with counterpart rats given a diet with soyabean meal)

| Bacterial status... | Conventional | Germ free |
|---|---|---|
| Reference... | Nugon-Baudon *et al.* (1990*a*) | Rabot *et al.* (1993*b*) |
| Duration of trial (weeks)... | 4 | 3 |
| No. of animals... | 11 | 8 |
| Cytochrome P450 | 75** | 80 |
| Glutathione-*S*-transferase | 236** | 105 |
| UDP-glucuronosyltransferase | 372** | 102 |

Mean values were significantly different from those for counterpart animals given a soyabean meal diet: ** $P < 0{\cdot}01$.

spontaneous mammary tumour mouse model, tumour incidence and multiplicity were significantly reduced after mice had received a diet containing 500 or 2000 mg/kg I3C for 8 months; in this model, I3C increased the level of oestradiol 2-hydroxylation up to 5-fold. The authors concluded that the protective effect may have resulted from increased 2-hydroxylation and inactivation of endogenous oestrogens (Bradlow *et al.* 1991). This could be a clue that GSL influence on carcinogenesis might result from alterations of the XME mediated biotransformation of exogenous compounds as well as endogenous molecules.

Finally, all pure GSL derivatives examined so far, in cell cultures and *in vivo*, originate from hydrolysis by plant myrosinase. Since GSL derivatives produced by myrosinase-like activities of the intestinal microflora are able to induce toxic effects, one wonders whether they are able to induce XME alterations as well. A first answer arises from experiments performed in our laboratory using conventional and germ free rats: the decrease in total P450 concentration as well as the induction of GST and GT observed in the liver of conventional rats given a GSL-rich but myrosinase free diet cannot be reproduced in germ free animals (Table 7; Nugon-Baudon *et al.* 1990*a*; Rabot *et al.* 1993*b*). These findings indicate that, should myrosinase be absent from the diet, bacterial metabolism would substitute for it and produce GSL derivatives capable of altering the XME system. Primary GSL derivatives produced by plant myrosinase or by the microflora may also undergo further transformations in the body. Whether or not these putative second metabolic steps are mediated by the intestinal microflora, one may think that the active GSL metabolites may not be exclusively the aglucones released by the plant myrosinase or the primary metabolites released by the microflora. We have been able to offer some support for this hypothesis (Nugon-Baudon *et al.* 1990*a*) by showing that a pretreatment with phenobarbital led to enhancement of several GSL linked toxic effects in conventional rats.

On the whole there is still a tremendous and varied scope for further research in the field of relationships between glucosinolates and cancer. The numerous studies already performed and the hypotheses already suggested demonstrate the challenge to the imagination and ingenuity of nutritionists, pharmacologists, chemists and bacteriologists posed by the puzzle.

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