Rape-seed meal toxicity in gnotobiotic rats: influence of a whole human faecal flora or single human strains of *Escherichia coli* and *Bacteroides vulgatus*

BY SYLVIE RABOT, LIONELLE NUGON-BAUDON, PIERRE RAIBAUD AND ODETTE SZYLIT

Unité d'Ecologie et de Physiologie du Système Digestif, Centre de Recherches de Jouy, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas Cédex, France

(*Received 6 January 1992 – Accepted 7 September 1992*)

Gnotobiotic growing rats harbouring either a whole human faecal flora or single human strains of *Escherichia coli* (EM0) or *Bacteroides vulgatus* (BV8H1) were fed for 7 weeks on semi-synthetic diets in which the protein source was either soya-bean meal (SM) or rape-seed meal (RM). For each bacterial status the RM-diet group was compared with the control group fed on the SM diet. The association of human faecal flora with the RM diet was responsible for reduced feed intake and reduced weight gain, an enlargement of the liver and thyroid and a decrease in both thyroxine and triiodothyronine plasma levels. The association of the *B. vulgatus* BV8H1 strain with the RM diet reproduced all these effects, except that triiodothyronine plasma levels were not significantly modified. Rats inoculated with the *E. coli* EM0 strain and fed on the RM diet exhibited a goitre and lowered thyroxine and triiodothyronine plasma levels. These results show that the human intestinal microflora may be involved in glucosinolate metabolism when cruciferous vegetables are consumed by man. The specificity of the symptoms observed according to the rat bacterial status supports the hypothesis that bacteria yield specific toxic glucosinolate derivatives according to their enzymic potential.

Rape-seed meal: Gnotobiotic rats: Human intestinal flora

Cruciferous plants (cabbage, turnip, Brussels sprouts, cauliflower, rape etc.) are commonly present in human and animal diets. They contain glucosinolates (thioglycoside compounds) that are hydrolysed by vegetal or bacterial thioglycosidases (myrosinases) into derivatives responsible for undesirable side-effects such as depletion of feed intake and growth rate, enlargement of target organs (liver, kidneys, thyroid glands), liver haemorrhages and thyroid hormonal disorders (Fenwick *et al.* 1983; Bell, 1984). The nature and the severity of these symptoms vary according to the animal species (Bourdon *et al.* 1981; Butler *et al.* 1982; Vermorel *et al.* 1987, 1988).

In man, epidemiological surveys show a correlation between endemic goitre and the consumption of important amounts of cruciferous vegetables or milk from cows fed on brassicaceous crops (Clements, 1955; Michajlovskij *et al.* 1969; Mitjavila, 1986). Moreover, experimental studies show that the ingestion of cruciferous vegetables or of glucosinolates or glucosinolate derivatives can significantly reduce radioactive I uptake by the thyroid glands (Greer & Astwood, 1948; Langer *et al.* 1971). However, other findings do not indicate thyroid troubles in volunteers fed on Brussels sprouts (McMillan *et al.* 1986).

In contrast to these undesirable properties, feeding cruciferous vegetables or glucosinolate derivatives can modify endogenous detoxication processes (McDanell *et al.* 1989; Nugon-Baudon *et al.* 1990*a*) and thus, may interfere in a positive way with the

metabolism of chemical carcinogens (Stoewsand *et al.* 1988) or more generally of toxic chemical compounds.

These phenomena should receive consideration since cruciferous intakes, which vary considerably according to geographical region, sex, age-group or income, may be associated with ingestion of large amounts of glucosinolates in some circumstances (Benns *et al.* 1978; Mullin & Sahasrabudhe, 1978; Sones *et al.* 1984).

Greer & Deeney (1959) were the first to suggest a role for the intestinal microflora in glucosinolate hydrolysis *in vivo*. This hypothesis was later reinforced by Oginsky *et al.* (1965) who showed that human faecal bacterial strains belonging to the Enterobacteriaceae were able to convert progoitrin into goitrin *in vitro*. Experiments using germ-free and conventional rats and chickens fed on rape-seed-meal-based diets gave definitive evidence of the responsibility of the intestinal flora in the release of glucosinolate derivatives *in vivo* (Nugon-Baudon *et al.* 1988); indeed, whereas both conventional rats and chickens fed on the rape-seed-meal-based diet exhibited usual glucosinolate-linked symptoms, no toxic effect was observed in their germ-free counterparts receiving the same diet; for each bacterial status, animals receiving a glucosinolate-free diet were used as controls. Furthermore, each of the whole microflora sources has specific effects when determined using gnotobiotic rats harbouring a whole chicken flora (Nugon-Baudon *et al.* 1988). Recently, a *Lactobacillus* strain isolated from the crop of a chicken was shown to induce a goitre in gnotobiotic rats fed on a rape-seed-meal diet (Nugon-Baudon *et al.* 1990*b*). However, most of the bacteria involved in these phenomena remain unidentified.

The initial aim of the present work was to study the effects of cruciferous vegetables in gnotobiotic rats harbouring a whole human faecal flora. This mimetic model is indeed an excellent simulator of what could happen in a human digestive tract (Mallett *et al.* 1987; Debure *et al.* 1989). Thereafter, we looked for specific myrosinase (*EC* 3.2.3.1)-like activity of individual bacterial strains belonging to this flora, challenging them in gnotobiotic rats fed on a cruciferous vegetable-based diet. Therefore, two strains from our laboratory collection were chosen: the first strain was an *Escherichia coli* strain, since Enterobacteriaceae strains have previously been demonstrated to have activity towards progoitrin *in vitro* (Oginsky *et al.* 1965); the second strain was a *Bacteroides vulgatus* strain, as it belongs to the predominant species of the human faecal flora (Finegold *et al.* 1983).

EXPERIMENTAL

Experimental diets

Two semi-synthetic diets were used, being isonitrogenous and isoenergetic (Table 1). In one diet the protein fraction was supplied by soya-bean meal (SM diet). In the other, soya-bean meal was substituted by rape-seed meal (RM diet). Dehulled 00 rape-seed meal (Darmor) containing 36·7 μmol glucosinolates/g dry matter was supplied by Centre Technique Interprofessionnel des Oléagineux Métropolitains, Paris, France. Pelleted diets packed in double-vacuum bags were sterilized by irradiation at 40 kGy. The glucosinolate content of the irradiated rape-seed meal was determined using a GLC method (Centre Technique Interprofessionnel des Oléagineux Métropolitains, 1987) (Table 2).

Animals: inoculation and maintenance

Three groups of twelve male germ-free Fischer 344 rats weighing about 80 g at the beginning of trials were used. Each group was housed in separate Trexler-type isolators fitted with a rapid transfer system (La Calhène, Vélizy, France).

Whole faecal flora from a healthy adult male subject was administered orally (oesophagal tubing) to each rat in the first group (HFF rats), using 1 ml of freshly passed stools (10 g/l;

Table 1. *Composition of diets (g/kg)*

Protein source...	Rape-seed meal	Soya-bean meal
Rape-seed meal (Darmor 00)	390·00	—
Soya-bean meal	—	276·00
Maize starch	531·40	576·00
Maize oil	20·00	20·00
Lysine hydrochloride	0·60	—
Vitamin mixture	18·00	18·00
Mineral mixture	40·00	40·00
Cellulose	—	70·00
Protein (N × 6·25) (g/kg dry matter)	145·00	150·00
ME (kJ/kg dry matter)	15270	15440

ME, metabolizable energy

Table 2. *Glucosinolate (GLS) content of irradiated Darmor rape-seed meal (μmol/g dry matter)*

Dry matter content (g/kg)	917
Progoitrin	23·9
Gluconapoleiferin	1·2
Gluconapin	6·3
Glucobrassicanapin	1·6
Sinalbin	—
Gluconasturtin	—
4-hydroxy-glucobrassicin	3·2
Glucobrassicin	0·5
Neo-glucobrassicin	—
4-methoxy-glucobrassicin	—
Total alkenyls	33·0
Total indoles	3·7
Total GLS	36·7

prepared in an anaerobic chamber (Aranki *et al.* 1969) and transferred into the isolator in a butyl-rubber-stoppered tube). An *E. coli* strain (EM0) (Duval-Iflah *et al.* 1981) was administered orally to each rat in the second group using 1 ml of an overnight aerobic culture on a liquid medium, pH 7·0, containing (g/l): tryptone (Difco, Detroit, MI, USA) 5, nutrient broth (Difco) 5, yeast extract (Difco) 1, NaCl 5. A *B. vulgatus* strain (BV8H1) was administered orally to each rat in the third group, using 1 ml of a 24 h anaerobic culture on a brain–heart infusion (Difco), pH 7·0, with added yeast extract (Difco) 5 g/l, and hemin (Sigma, La Verpillière, France) 5 mg/l. For each bacterial status, animals were then randomly divided into two subgroups of six animals each; one group received the control diet (SM diet) and the other group received the RM diet. Animals were fed *ad lib.* and were given sterilized (20 min, 120°) tap-water to drink.

The room temperature was 21° with a 12 h light–dark cycle. Animal weight and feed intake were measured once weekly for 7 weeks.

Bacteriological methods

Bacterial population levels in rats inoculated with a single strain were measured weekly by culture of faecal samples serially diluted 10-fold in a liquid medium, pH 7·0, containing (g/l) casein enzymic-hydrolysate (USBC, Cleveland, Ohio) 2, yeast extract (Difco) 2, NaCl 5, KH_2PO_4 1. *E. coli* EM0 was grown aerobically for 24 h at 37° on an agar medium, pH 7·0, containing (g/l): tryptone (Difco) 5, nutrient broth (Difco) 5, yeast extract (Difco) 1, NaCl 5, agar (Touzart et Matignon, Vitry-sur-Seine, France) 10. *B. vulgatus* BV8H1 was grown anaerobically for 48 h at 37° on brain–heart infusion (Difco), pH 7·0, with added yeast extract (Difco) 5 g/l, hemin (Sigma) 5 mg/l, Bitek agar (Difco) 14 g/l.

Sample collection

The animals were anaesthetized with chloroform 7 weeks after inoculation. Blood samples were collected by cardiac puncture in order to obtain a sample of plasma which was stored at −20° until analysed. Rats were then killed using chloroform and the glucosinolate target organs (liver, kidneys and thyroid glands) were removed quickly and weighed after removal of surrounding fat. For groups inoculated with a single species the gnotobiotic status and the bacterial population levels in the caecal contents were checked as already described for faecal samples (see p. 326).

Hormone assays

Plasma tetraiodothyronine (T4) and triiodothyronine (T3) concentrations were determined in duplicate using an ELISA method (Immunodiagnostics Enzymun Tests T4 and T3; Boehringer Mannheim, Meylan, France). These Enzymun kits, originally designed for use with human plasma, have been used previously (Nugon-Baudon *et al.* 1990*a*, *b*); data obtained for rats using these kits are consistent with those reported by other authors using different methods (Ukai & Mitsuma, 1977; Jordan *et al.* 1980; Vermorel *et al.* 1986, 1988).

Statistical analyses

Bacteriological data are expressed as $\log_{10}$ of the bacterial faecal or caecal populations. Anatomical data are expressed as g or mg/kg body weight.

Results are expressed as means with their standard errors. For each bacterial status, the RM-diet group was compared with the control group fed on the SM diet using Student's *t* test. Statistical significance was at $P < 0{\cdot}01$ level.

RESULTS

Caecal bacterial counts in rats inoculated with a single species

Whatever the strain, the RM diet did not significantly modify the caecal population levels since caecal counts were 9·7 (SE 0·7) *v.* 10·2 (SE 0·2) for EM0–SM-fed rats and EM0–RM-fed rats respectively, and 9·1 (SE 0·2) *v.* 9·7 (SE 0·2) for BV8H1–SM-fed rats and BV8H1–RM-fed rats respectively.

Effects of RM diet on growth curve and feed intake in relation to the bacterial status

When HFF rats were fed on the RM diet, growth rate was dramatically decreased from the second week post-inoculation (Table 3). Cumulative weight gain at the end of the experiment was 56% lower in this group compared with the control group fed on the SM diet. This was correlated with a reduced feed intake: 10 g/rat per d *v.* 19 g/rat per d in the RM and SM groups respectively, as calculated from the intake values for the whole group.

In rats harbouring the EM0 strain, growth rates were similar whatever the diet (Table 4). Feed intake was 15 g/rat per d *v.* 19 g/rat per d in the RM and SM groups respectively.

Table 3. *The effect of diet† on growth curve, target organ weight and thyroid hormones in gnotobiotic rats harbouring a whole human faecal flora‡*

(Mean values with their standard errors)

	Diet			
No. of animals...	Soya-bean meal 6		Rape-seed meal 6	
	Mean	SE	Mean	SE
Initial body wt (g)	82	13	81	13
Cumulative wt gain (g)				
Time (weeks)				
1	29	2	29	2
2	68	2	42***	2
3	97	4	47***	2
4	132	4	55***	4
5	152	7	62***	7
6	178	7	73***	11
7	181	7	79***	13
Liver (g/kg body wt)	38·2	1·1	45·1***	2·0
Kidneys (g/kg body wt)	8·2	0·4	8·5	0·9
Thyroid (mg/kg body wt)	36	2·0	169***	9
Tetraiodothyronine (nmol/l plasma)	54·9	14·3	28·6**	5·1
Triiodothyronine (nmol/l plasma)	1·71	0·16	1·33**	0·20

Mean values were significantly different from those for soya-bean-meal diet: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.
† For details of diets, see p. 324 and Tables 1 and 2.
‡ For details of experimental procedures, see pp. 324–326.

On the contrary, growth rate was strongly decreased in BV8H1-treated rats fed on the RM diet compared with their SM counterparts (Table 5). Depletion of the weight gain at the end of trials reached 72%. This result was correlated with a reduced feed intake: 9 g/rat per d *v.* 19 g/rat per d in the RM and SM groups respectively.

Effects of the RM diet on target organ weights in relation to bacterial status

Compared with their SM counterparts, HFF–RM-fed rats exhibited an enlargement of the liver (+18%) and thyroid (+369%), whereas no effect was seen on kidney weight (Table 3).

In gnotobiotic rats, the effects of the RM diet on target organ weights were different according to the strain: rats harbouring the EM0 strain only suffered from a thyroid enlargement (+202%) (Table 4), whereas inoculation with the BV8H1 strain led to a dramatic goitre (+572%) and an enlargement of the liver (+14%; Table 5). Kidney weight was not modified in any case.

Effects of the RM diet on thyroid hormones in relation to the bacterial status

A significant decrease in both T4 and T3 plasma concentrations (−48 and −22% respectively) was observed in the HFF–RM-fed group compared with its SM counterpart (Table 3).

In the same way, T4 and T3 plasma levels were respectively 44 and 29% lower in the EM0–RM-fed group than in the EM0–SM-fed group (Table 4).

Table 4. *The effect of diet† on growth curve, target organ weight and thyroid hormones in gnotobiotic rats harbouring a human strain of* Escherichia coli‡

(Mean values with their standard errors)

No. of animals...	Diet			
	Soya-bean meal 6		Rape-seed meal 6	
	Mean	SE	Mean	SE
Initial body wt (g)	79	4	75	4
Cumulative wt gain (g)				
Time (weeks)				
1	19	4	34	2
2	63	4	69	4
3	95	7	89	7
4	130	9	114	11
5	163	11	137**	9
6	175	9	155	11
7	184	9	165	9
Liver (g/kg body wt)	40·1	0·2	40·4	1·1
Kidneys (g/kg body wt)	7·7	0·4	7·3	0·7
Thyroid (mg/kg body wt)	48	5	145***	18
Tetraiodothyronine (nmol/l plasma)	55·7	4·7	31·0***	2·2
Triiodothyronine (nmol/l plasma)	1·43	0·18	1·01**	0·20

Mean values were significantly different from those for soya-bean-meal diet: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

† For details of diets, see p. 324 and Tables 1 and 2.

‡ For details of experimental procedures, see pp. 324–326.

As for the BV8H1-treated rats, a significant depletion in T4 levels occurred in the RM-group (−43%); the T3 concentration was also strongly decreased (−29%) but this effect was not significant (Table 5).

DISCUSSION

The whole human faecal flora, as well as single strains isolated from it, displays a myrosinase-like potential when implanted in the rat digestive tract. Thus, it may be assumed that the intestinal microflora could be involved in the thyroid disorders sometimes observed in humans consuming cruciferous vegetables (Clements, 1955; Michajlovskij *et al.* 1969; Mitjavila, 1986).

Furthermore, changes in feed intake and growth curve and liver hypertrophy observed in HFF rats, as well as in rats inoculated with the BV8H1 strain, show that this myrosinase-like potential is not restricted to the development of thyroid disorders. The discrepancies between these effects and what has been so far described in man could arise from the origin and the high level of glucosinolates used in our experimental diets, which are different from what is usually encountered in human diets. Actually these experimental conditions, i.e. a high level of glucosinolates always supplied by the same source of cruciferous vegetables, were chosen to favour as high bacterial myrosinase-like activities as possible and to compare them whatever the origin of the flora (rat, chicken, man etc.) harboured by the gnotobiotic rats (Nugon-Baudon *et al.* 1988).

HFF–RM-fed rats exhibited symptoms different from those observed in conventional rodents (Vermorel *et al.* 1987; Nugon-Baudon *et al.* 1988, 1990*a*). Indeed we did not

Table 5. *The effect of diet*† *on growth curve, target organ weight and thyroid hormones in gnotobiotic rats harbouring a human strain of* Bacteroides vulgatus‡

(Mean values with their standard errors)

	Diet			
No. of animals...	Soya-bean meal 6		Rape-seed meal 6	
	Mean	SE	Mean	SE
Initial body wt (g)	109	2	116	4
Cumulative wt gain (g)				
Time (weeks)				
1	24	2	30	4
2	60	4	44***	2
3	99	7	42***	2
4	134	9	45***	7
5	158	9	49***	4
6	175	9	48***	7
7	187	11	53***	9
Liver (g/kg body wt)	35·6	0·9	40·7***	1·1
Kidneys (g/kg body wt)	7·1	0·4	8·0	0·7
Thyroid (mg/kg body wt)	36	2	242***	20
Tetraiodothyronine (nmol/l plasma)	71·3	6·7	40·6**	12·3
Triiodothyronine (nmol/l plasma)	1·44	0·16	1·02	0·49

Mean values were significantly different from those for soya-bean-meal diet: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

† For details of diets, see p. 324 and Tables 1 and 2.

‡ For details of experimental procedures, see pp. 324–326.

reproduce hypertrophy of the kidneys. Therefore, it is likely that the discrepancies in undesirable effects related to the animal species do not result from a host-related sensitivity but from the nature of the intestinal microflora. This confirms the conclusion from a previous experiment comparing conventional rats with rats harbouring a whole chicken microflora (Nugon-Baudon *et al.* 1988).

In rats inoculated with either the EM0 strain or the BV8H1 strain the caecal population levels were high and were not modified by the protein source of the diet. The toxic side-effects of the RM diet differed according to the bacterial strain. The association of the BV8H1 strain with the RM diet reproduced all the effects observed in HFF–RM-fed rats; however, mean T3 plasma concentrations in the BV8H1–SM-fed group and the BV8H1–RM-fed group were not significantly different, due to the large variation in the values for the RM group. The association of the EM0 strain with the RM diet only reproduced some of the findings obtained for HFF–RM-fed rats, i.e. a dramatic goitre associated with hypothyroidism. This is in agreement with the results of Oginsky *et al.* (1965), who demonstrated an *in vitro* conversion of progoitrin into goitrin by members of the family Enterobacteriaceae, since goitrin is thought to be the major antithyroid compound in cruciferous vegetables (Greer, 1962; Mitjavila, 1986). Moreover, since T4 and T3 plasma concentrations were strongly reduced in EM0–RM-fed rats whereas growth rate was not altered, the relationships between the decrease in weight gain and thyroid disorders are probably less exclusive and more complex than generally assumed. Thus, specific but still unknown bacterial glucosinolate derivatives may be involved in the decrease in weight gain of BV8H1–RM-fed rats.

In conclusion, the specificity of the symptoms in relation to the bacterial status of the rat supports the hypothesis that bacteria yield specific toxic glucosinolate derivatives in relation to specific enzymic properties. These findings are a new step in the exploration of the mechanisms involved in glucosinolate metabolism.

This work was supported by grants from the French Ministry of Agriculture and Forestry and from Centre Technique Interprofessionel des Oléagineux Métropolitains (CETIOM), Paris, France. The authors thank CETIOM for providing the Darmor rape-seed meal and Dr Ribaillier and his co-workers (CETIOM, Ardon, France) for glucosinolate analysis of the rape-seed meal. They are grateful to Dr Nicoli (Instituto de Ciencias Biológicas, Belo Horizonte, Brazil) for kindly isolating and providing the *B. vulgatus* BV8H1 strain.

REFERENCES

Aranki, A., Syed, S. A., Kenney, E. B. & Freter, R. (1969). Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. *Applied Microbiology* **17**, 568–576.

Bell, J. M. (1984). Nutrients and toxicants in rapeseed meal: a review. *Journal of Animal Science* **58**, 996–1010.

Benns, G. B., Hall, J. W. & Beare-Rogers, J. L. (1978). Intake of brassicaceous vegetables in Canada. *Canadian Journal of Public Health* **69**, 64–66.

Bourdon, D., Perez, J. M. & Baudet, J. J. (1981). Utilisation de nouveaux types de tourteaux de colza par le porc en croissance-finition: influence des glucosinolates et du dépelliculage (New types of rapeseed meal fed to growing-finishing pigs: influence of glucosinolates and dehulling). *Journées de la Recherche Porcine en France* **13**, 163–178.

Butler, E. J., Pearson, A. W. & Fenwick, G. R. (1982). Problems which limit the use of rapeseed meal as a protein source in poultry diets. *Journal of the Science of Food and Agriculture* **33**, 866–875.

Centre Technique Interprofessionnel des Oléagineux Métropolitains (1987). *Analyse des graines oléagineuses* (Analysis of oilseeds). Guide pratique AFNOR. Paris: CETIOM.

Clements, F. W. (1955). A thyroid blocking agent as a cause of endemic goitre in Tasmania: preliminary communication. *Medical Journal of Australia* **3**, 369–371.

Debure, A., Colombel, J. F., Flourie, B., Rautureau, M. & Rambaud, J. C. (1989). Comparaison de l'implantation et de l'activité métabolique d'une flore fécale de rat et d'une flore fécale humaine inoculées chez le rat axénique (Implantation and metabolic activity of rat and human fecal bacterial flora administered to germ-free rats). *Gastroentérologie Clinique et Biologique* **13**, 25–31.

Duval-Iflah, Y., Raibaud, P. & Rousseau, M. (1981). Antagonisms among isogenic strains of *Escherichia coli* in the digestive tracts of gnotobiotic mice. *Infection and Immunity* **34**, 957–969.

Fenwick, G. R., Heaney, R. K. & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *Critical Reviews in Food Science and Nutrition* **18**, 123–201.

Finegold, S. M., Sutter, V. L. & Mathisen, G. E. (1983). Normal indigenous intestinal flora. In *Human Intestinal Microflora in Health and Disease*, pp. 3–31 [D. J. Hentges, editor]. New York: Academic Press.

Greer, M. A. (1962). The natural occurrence of goitrogenic agents. *Recent Progress in Hormone Research* **18**, 187–219.

Greer, M. A. & Astwood, E. B. (1948). The antithyroid effect of certain foods in man as determined with radioactive iodine. *Endocrinology* **43**, 105–119.

Greer, M. A. & Deeney, J. M. (1959). Antithyroid activity elicited by the ingestion of pure progoitrin, a naturally occurring thioglycoside of the turnip family. *Journal of Clinical Investigation* **38**, 1465–1474.

Jordan, D., Rousset, B., Perrin, F., Fournier, M. & Orgiazzi, J. (1980). Evidence for circadian variations in serum thyrotropin, 3,5,3′-triiodothyronine, and thyroxine in the rat. *Endocrinology* **107**, 1245–1248.

Langer, P., Michajlovskij, N., Sedlak, J. & Kutka, M. (1971). Studies on the antithyroid activity of naturally occurring L-5-vinyl-2-thiooxazolidone in man. *Endokrinologie* **57**, 225–229.

McDanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K. & Fenwick, G. R. (1989). The effect of feeding brassica vegetables and intact glucosinolates on mixed-function oxidase activity in the livers and intestines of rats. *Food and Chemical Toxicology* **27**, 289–293.

McMillan, M., Spinks, E. A. & Fenwick, G. R. (1986). Preliminary observations on the effect of dietary Brussels sprouts on thyroid function. *Human Toxicology* **5**, 15–19.

Mallett, A. K., Bearne, C. A., Rowland, I. R., Farthing, M. J. G., Cole, C. B. & Fuller, R. (1987). The use of rats associated with a human faecal flora as a model for studying the effects of diet in the human gut microflora. *Journal of Applied Bacteriology* **63**, 39–45.

Michajlovskij, N., Sedlak, J., Jusic, M. & Buzina, R. (1969). Goitrogenic substances of kale and their possible relations to the endemic goitre on the island of Krk (Yugoslavia). *Endocrinologia Experimentalis* **3**, 65–72.

Mitjavila, S. (1986). Substances naturelles nocives des aliments (Natural toxic compounds in food). *Toxicologie et Sécurité des Aliments*, pp. 129–157. Paris: Lavoisier et Apria.

Mullin, W. J. & Sahasrabudhe, M. R. (1978). An estimate of the average daily intake of glucosinolates via cruciferous vegetables. *Nutrition Reports International* **18**, 273–279.

Nugon-Baudon, L., Rabot, S., Szylit, O. & Raibaud, P. (1990*a*). Glucosinolate toxicity in growing rats: interactions with the hepatic detoxification system. *Xenobiotica* **20**, 223–230.

Nugon-Baudon, L., Rabot, S., Wal, J. M. & Szylit, O. (1990*b*). Interactions of the intestinal microflora with glucosinolates in rapeseed meal toxicity: first evidence of an intestinal *Lactobacillus* possessing a myrosinase-like activity *in vivo*. *Journal of the Science of Food and Agriculture* **52**, 547–559.

Nugon-Baudon, L., Szylit, O. & Raibaud, P. (1988). Production of toxic glucosinolate derivatives from rapeseed meal by intestinal microflora of rat and chicken. *Journal of the Science of Food and Agriculture* **43**, 299–308.

Oginsky, E. L., Stein, A. E. & Greer, M. A. (1965). Myrosinase activity in bacteria as demonstrated by the conversion of progoitrin to goitrin. *Proceedings of the Society for Experimental Biology and Medicine* **119**, 360–364.

Sones, K., Heaney, R. K. & Fenwick, G. R. (1984). An estimate of the mean daily intake of glucosinolates from cruciferous vegetables in the UK. *Journal of the Science of Food and Agriculture* **35**, 712–720.

Stoewsand, G. S., Anderson, J. L. & Munson, L. (1988). Protective effect of dietary Brussels sprouts against mammary carcinogenesis in Sprague–Dawley rats. *Cancer Letters* **39**, 199–207.

Ukai, M. & Mitsuma, T. (1977). Plasma triiodothyronine, thyroxine and thyrotrophin levels in germfree rats. *Experientia* **34**, 1095–1096.

Vermorel, M., Davicco, M. J. & Evrard, J. (1987). Valorization of rapeseed meal. 3. Effects of glucosinolate content on food intake, weight gain, liver weight and plasma thyroid hormone levels in growing rats. *Reproduction Nutrition Développement* **27**, 57–66.

Vermorel, M., Heaney, R. K. & Fenwick, G. R. (1986). Nutritive value of rapeseed meal: effects of individual glucosinolates. *Journal of the Science of Food and Agriculture* **37**, 1197–1202.

Vermorel, M., Heaney, R. K. & Fenwick, G. R. (1988). Antinutritional effects of the rapeseed meals, Darmor and Jet Neuf, and progoitrin together with myrosinase, in the growing rat. *Journal of the Science of Food and Agriculture* **44**, 321–334.

Printed in Great Britain