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The Fate of Intense Sweeteners in the Body

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ABSTRACT

Four intense sweeteners have been, or are likely to be, used extensively in Europe. Saccharin and acesulfam-K are acidic cyclic sulphonamides which are absorbed well from the gut and eliminated in the urine without undergoing detectable metabolism. The increase in bladder tumours detected when male rats are fed high dietary levels of saccharin from birth cannot be explained simply by accumulation of the sweetener in the bladder. Cyclamate is metabolized by the gut microflora to cyclohexylamine, which is more toxic than the parent sweetener. The extent of metabolism shows very wide inter- and intra-individual variability. Aspartame is a dipeptide derivative which is hydrolyzed in the intestine. Thus the potential for toxicity is related to its metabolites, phenylalanine, aspartic acid and methanol.

INTRODUCTION

This review will consider four intense sweeteners which have been used as food additives in the UK (Fig. 1). They will be discussed in the chronological order in which they appeared on the market, i.e. saccharin, cyclamate, aspartame and acesulfam-K. Their fate in the body will be considered in relation to the available toxicity data and to their safety in use.

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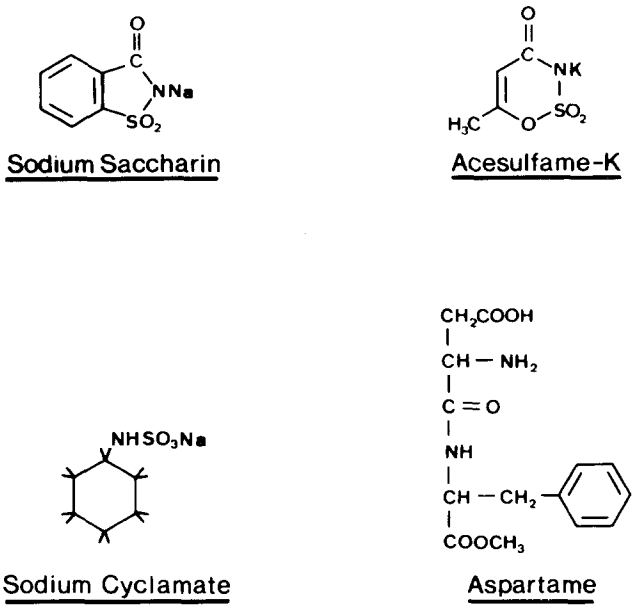

Fig. 1. The intense sweeteners considered in this paper.

SACCHARIN

Saccharin has been used as a non-nutritive sweetener since the turn of this century; however, its safety as a food additive is a matter of considerable debate. Administration of high dietary levels of saccharin (3 % or more) to male rats from birth results in an increased incidence of tumours of the urinary bladder (Arnold *et al.*, 1980; Taylor *et al.*, 1980; Geil, 1983). However, the relevance of these findings to levels of use in man is questioned by the failure of a number of large epidemiology studies to demonstrate a consistent increased risk of bladder cancer associated with the use of artificial sweeteners. (Burbank & Fraumeni, 1970; Armstrong & Doll, 1975; Howe *et al.*, 1977; Wynder & Goldsmith, 1977; Kessler & Clark, 1978; Hoover & Strasser, 1980; Morrison & Buring, 1980; Wynder & Stellman, 1980; Cartwright *et al.*, 1981; Jensen & Kamby, 1982; Morrison *et al.*, 1982; Najem *et al.*, 1982). Thus, any consideration of the fate of saccharin in the body should concentrate on the effect of dose on the disposition of saccharin and also a comparison of its fate in rat and

man. Fortunately, the published data on the fate of saccharin are sufficiently extensive to allow a detailed analysis. The various studies on the fate of saccharin in the body are important in assessment of the most suitable directions for future research into the mechanisms by which feeding high dietary levels increases the incidence of tumours of the urinary bladder in male rats.

Absorption from the gut

A review of the literature shows that saccharin is incompletely absorbed after oral administration to rats or man. In the rat, about 20–30% of an oral dose (5–500 mg kg^{-1}) of radio-labelled saccharin is recovered in the faeces (Table 1). The low faecal elimination of a tracer dose (0·06 mg kg^{-1}) suggests that absorption may be dose-dependent at very low doses (< 5 mg kg^{-1}). Saccharin was absorbed more completely from the gut of man, since only about 5% of a radio-labelled dose (0·2–20 mg kg^{-1}) was recovered in the faeces. In contrast to these oral data, negligible amounts of saccharin (about 2%) were recovered in the faeces following intravenous administration to rats (Sweatman and Renwick, 1980).

The incomplete absorption of saccharin from the gut, combined with its rapid excretion (see later), results in the plasma concentration/time curve after oral dosing being determined by the absorption of the sweetener from the gut of both rat (Sweatman & Renwick, 1980) and man (Sweatman *et al.*, 1981).

TABLE 1
Absorption of Saccharin

Species	*Dose* (*mg kg*$^{-1}$)	*% in faeces*	*Reference*
Rat	0·06	3 ± 2	Sweatman & Renwick (1979)
	16–22	16 (6–22)	Ball *et al.* (1977)
	300	26 (24–27)	Minegishi *et al.* (1972)
	5% in diet	14 ± 5	Sweatman & Renwick (1979)
	5	22	Lethco & Wallace (1975)
	50	17	Lethco & Wallace (1975)
	500	37	Lethco & Wallace (1975)
Man	0·2	3 (2–4)	Ball *et al.* (1977)
	7	6 ± 1	Byard *et al.* (1974)
	21	3 (0–8)	Sweatman *et al.* (1981)

Tissue distribution

Studies using single and multiple doses of radio-labelled saccharin have shown that the highest concentrations were in the organs of excretion, the kidney and bladder, with lower levels in the plasma and other tissues (Matthews *et al.*, 1973; Pitkin *et al.*, 1971*b*; Ball *et al.*, 1977). The concentrations in the kidney and bladder showed high variability, but suggested that saccharin may be cleared more slowly from the bladder than from other tissues (Matthews *et al.*, 1973). This was even more apparent for the foetal bladder (Ball *et al.*, 1977) and, at the time of the National Academy of Sciences Review of Saccharin in 1978 (National Academy of Science, 1978), it seemed possible that the bladder may have been the target due to unique accumulation of saccharin within the tissue.

In contrast to these single- and multiple-dose radio-labelled studies, measurements of the concentrations of saccharin in the tissues of male rats fed diets containing 1–10% saccharin (Sweatman & Renwick, 1980) failed to show significant excessive accumulation in the bladder compared with other tissues. The concentration in the bladder tissue was only about 1·6 times that in plasma, whilst the concentration in the kidney was 3 times the corresponding plasma levels. The concentrations in the tissues of female rats fed a saccharin diet were similar to those in males, and there was no evidence of a marked difference between F_0 and F_1 animals (Sweatman & Renwick, 1982). Thus the organ and sex differences in sensitivity to tumour development, and the requirement for the administration of saccharin diets from birth, do not correlate with a unique accumulation of saccharin within the target.

An interesting phenomenon observed during these tissue distribution studies was a non-linear relationship between the concentration of saccharin in the diet and that in plasma. Excessive accumulation in plasma and various organs occurred at dietary concentrations of 5% or more (Sweatman & Renwick, 1980). The cause of this was decreased renal clearance (see later).

The apparent volume of distribution following intravenous administration to rat (about 300–400 ml kg^{-1}) (Sweatman & Renwick, 1980) and man (about 280 ml kg^{-1}) (Sweatman *et al.*, 1981) is consistent with the relatively lower concentration found in most tissues compared with plasma.

Metabolism

Early metabolism studies using radio-labelled saccharin reported the presence of traces of hydrolysis products (2-sulphamoyl- and 2-sulphobenzoic acid (Pitkin *et al.*, 1971*a*; Kennedy *et al.*, 1972; Lethco and Wallace, 1975)). However, a number of more thorough studies of equal or greater accuracy and specificity have failed to reproduce these findings (Minegishi *et al.*, 1972; Byard & Golberg, 1973; Matthews *et al.*, 1973; Ball *et al.*, 1977; Sweatman & Renwick, 1979, 1980). No metabolites of saccharin have been reported in human subjects given [^{14}C] saccharin (Byard *et al.*, 1974; Ball *et al.*, 1977), (see Renwick (1983*a*) for a complete review).

The absence of detectable metabolism of saccharin is important. The sweetener is a nucleophilic molecule and thus metabolism would be necessary to produce an electrophilic species capable of acting as an alkylating type of initiator of carcinogenesis. Consistent with the absence of metabolism is a lack of covalent binding to DNA of the bladder *in vivo* (Lutz & Schlatter, 1977), a property which is characteristic of classical chemical carcinogens such as benzo[*a*]pyrene and nitrosamines.

Excretion

The main route of elimination of circulating saccharin is via the kidneys. Kidney slices are able to take up saccharin *in vitro* from the medium against a concentration gradient, the process being inhibited by PAH (Goldstein *et al.*, 1978; Bourgoignie *et al.*, 1980; Berndt *et al.*, 1981). *In vivo* the renal clearance exceeds that of inulin (Goldstein *et al.*, 1978; Bourgoignie *et al.*, 1980; Bekersky *et al.*, 1980) and is inhibited by the drug probenecid (Sweatman & Renwick, 1980), indicating that renal tubular secretion is a major excretory mechanism and may account for the removal of about 70% of circulating saccharin at low doses. In rats, the clearance of saccharin was dose-dependent following an intravenous bolus dose, intravenous infusion (Sweatman & Renwick, 1980) and following dietary administration at concentrations up to 10% (Sims & Renwick, 1983). Saturation of elimination occurred at plasma concentrations of saccharin greater than about 200 $\mu g\,ml^{-1}$. These levels were similar to those in rats showing excessive plasma and tissue accumulation of saccharin, suggesting that reduced elimination was the underlying cause of the non-linear disposition detected.

No saturation of renal elimination of saccharin was found in man following intravenous administration of $10\,mg\,kg^{-1}$ or single oral doses of 2 g (Sweatman *et al.*, 1981). However, the plasma levels did not exceed $100\,\mu g\,ml^{-1}$, even transiently, despite the very high dose taken.

Summary

Thus, within the confines of present day techniques it is possible to draw the following conclusions:

(a) saccharin is not metabolized to an electrophilic species capable of binding to DNA;
(b) the urinary bladder, like the kidney, contains higher concentrations of saccharin than other tissues, but there is no evidence of excessive accumulation on chronic administration;
(c) the feeding protocol and sex-selective sensitivity to tumour development is not due to specific accumulation in the urinary bladder of male rats given saccharin from birth;
(d) high dietary levels are not representative of low doses due to saturation of renal tubular secretion;
(e) the rat is a reasonable model for man, providing the dose used does not overload the normal excretory function of the kidney.

Thus, recent research on saccharin has concentrated on the metabolic and physiological effects produced by feeding high dietary concentrations of saccharin. Tumourigenicity studies have defined the dose response for bladder tumour formation and continue to explore the 'post-initiation' stages of tumour development at which saccharin may exert its effects.

CYCLAMATE

Cyclamic acid (cyclohexanesulphamic acid) is a strong organic acid and its sodium and calcium salts are extremely water soluble. Early studies (see Williams, 1959) showed that it was absorbed incompletely from the gut and eliminated from the body in the urine and faeces as unchanged cyclamate.

In 1966, Kojima & Ichibagase (1966) showed that it was metabolized in man and dog to cyclohexylamine, which is excreted rapidly in the urine but is more toxic than the parent compound. Thus, the extent of cyclohexylamine formation is an important determinant of the safe level

of use of the sweetener, which is still available in many countries around the world.

The induction of cyclamate metabolism

An important difference between early studies, which did not detect cyclohexylamine (Taylor *et al.*, 1951; Miller *et al.*, 1966), and later positive investigations, was the prior exposure of the test animal or individual to cyclamate. [^{14}C]Cyclohexylamine was detected in the urine of rats, rabbits and guinea pigs given [^{14}C]cyclamate following chronic cyclamate ingestion, but not in untreated animals (Renwick & Williams, 1972*b*).

In rats, the duration of chronic administration reported to be necessary before cyclamate metabolism can be detected is extremely variable, with some studies detecting cyclohexylamine in less than 3 months (Renwick & Williams, 1972*b*; Bickel *et al.*, 1974), whilst others did not detect metabolism until after 12 months chronic intake (Renwick, 1976) or not at all (Collings, 1971). Once the metabolizing ability has been established in a rat colony, it can be transferred from animal to animal and from cage to cage by coprophagy (Collings, 1971; Dalderup *et al.*, 1970).

Cyclohexylamine has been detected in the urine of man following a single dose of cyclamate (Glogner, 1970; Asahina *et al.*, 1971; Hengstmann *et al.*, 1971; Renwick & Williams, 1972*b*). However, excretion of cyclohexylamine is mostly in the second to fourth day after dosing, whereas cyclamate is excreted mainly during the first 48 h (Schoenberger *et al.*, 1953; Asahina *et al.*, 1971; Renwick & Williams, 1972*b*).

During chronic administration of cyclamate to man, the excretion of cyclohexylamine increases until a plateau is reached after approximately 4–10 days of continuous intake (Leahy *et al.*, 1967*a*; Davis *et al.*, 1969; Pawan, 1970; Collings, 1971; Renwick & Williams, 1972*b*). Continued administration is necessary for the maintenance of metabolizing activity, since removal of cyclamate from the diet results in a rapid loss in the ability to form cyclohexylamine in both rats and man (Renwick & Williams, 1972*b*; Bickel *et al.*, 1974).

The incidence of cyclamate metabolism

Not all individuals, whether rat or man, develop the ability to metabolise cyclamate and wide intersubject differences occur.

Several studies have analysed the urinary excretion of cyclohexylamine in large groups of individuals during the first 24 h following a single oral dose of cyclamate. Since this method would not analyse samples either during the maximum excretion of cyclohexylamine, or during chronic intake, the resulting data will tend to under-estimate the incidence of metabolism. A total of 480 subjects have been studied in this way and 76 (or 16%) excreted measurable levels of cyclohexylamine (Blumberg & Heaton, 1970; Glogner, 1970; Pawan, 1970; Hengstmann *et al.*, 1971; Litchfield & Swan, 1971).

Of far greater reliability is the measurement of cyclohexylamine in the urine of subjects who had received cyclamate regularly for at least 3 days before the urine collection. A total of 219 subjects have been studied using this approach, and 60 (or ¡27%) excreted measurable amounts of cyclohexylamine (Leahy *et al.*, 1967*a*,*b*; Wills *et al.*, 1968; Davis *et al.*, 1969; Collings, 1971; Renwick and Williams, 1972*b*).

The extent of cyclamate metabolism

Although 20–30% of the human population have the ability to metabolise cyclamate to cyclohexylamine, there are extremely wide intersubject differences in the extent of metabolism during chronic intake. Analysis of the published data on the extent of formation of cyclohexylamine in subjects given regular doses in the studies discussed above (Fig. 2) reveals that the majority metabolise less than 0·1% of the daily dose of cyclamate. About 90% of the population excrete less than 1% of a daily dose of cyclamate as cyclohexylamine. Such individuals are clearly at negligible risk compared with those who excrete larger amounts, i.e. about 4% of the population who excrete 20% or more as cyclohexylamine.

The site of metabolism

The site of cyclohexylamine formation in chronically treated animals has been shown to be the gut microflora. Evidence for this includes: (i) the extensive metabolism to cyclohexylamine *in vitro* by faeces and gut contents (Golberg *et al.*, 1969; Dalderup *et al.*, 1970; Asahina *et al.*, 1972*a*, *b*; Drasar *et al.*, 1972; Bickel *et al.*, 1974; Tesoriero & Roxon, 1975; Renwick, 1976) but not by the tissues (Prosky & O'Dell, 1971; Asahina *et al.*, 1972*b*; Drasar *et al.*, 1972) of known cyclamate metabolising animals; (ii) the negligible metabolism after parenteral

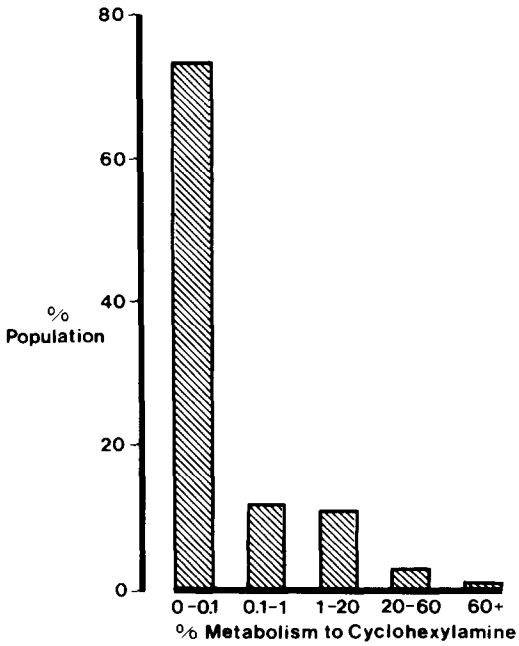

Fig. 2. The distribution of cyclamate metabolising ability in the human population during chronic intake for at least 3 days. The values are derived from all published data and represent a total of 221 individuals given 0·5–5 g of cyclamate day^{-1} (from Renwick, 1983*a*).

(systemic) administration compared with oral dosing in known converting animals (Sonders *et al.*, 1969; Collings, 1971; Renwick & Williams, 1972*b*; Bickel *et al.*, 1974); and (iii) the suppression of metabolism by oral administration of antibiotics (Sonders *et al.*, 1969; Collings, 1971; Bickel *et al.*, 1974). Studies implicating the liver as a possible site of metabolism (Kojima & Ichibagase, 1968; Ichibagase *et al.*, 1972) are difficult to interpret due to the extremely low levels of metabolism in the test animals, and the possible presence of cyclohexylamine in the tissues due to pre-treatment.

Evidence that the gut flora are also the site of cyclamate metabolism in man include: (i) the formation of cyclohexylamine on *in vitro* incubation of cyclamate with human faeces (Drasar *et al.*, 1972); (ii) the complete suppression of cyclamate metabolism by oral antibiotics (Collings, 1971); and (iii) the presence of *in vitro* metabolism in faeces collected during, but not preceding, chronic cyclamate intake, thus mirroring the fate *in vivo* (Drasar *et al.*, 1972).

Various strains of bacteria have been isolated from the faeces of human and animal cyclamate metabolizers, and shown to be able to form cyclohexylamine *in vitro*, e.g. both common intestinal organisms such as clostridia (Golberg *et al.*, 1969; Drasar *et al.*, 1972), enterobacteria and enterococci (Drasar *et al.*, 1972) and less common species such as *Pseudomonas* and *Corynebacterium* (Asahina *et al.*, 1972*b*). However, the mechanisms which control the development or induction of metabolism either *in vitro* or *in vivo* are at present unknown. The metabolizing system may be extremely complex, since it has been shown recently that four strains of bacteria isolated from guinea pigs could form cyclohexylamine if grown in combination, but not if incubated individually with cyclamate (Matsui *et al.*, 1981).

Metabolism and the establishment of an acceptable intake (ADI) for cyclamate

If the question of the carcinogenic potential of cyclamate is resolved, then the level of use of cyclamate will be determined by the toxic potential of the metabolite, cyclohexylamine (CHA). Once the maximum no-effect level (NOEL) for cyclohexylamine is determined, then the regulation of cyclamate will be possible by fitting appropriate values into the following equation:

$$\text{ADI for cyclamate} = \frac{\text{NOEL for CHA}}{\text{Safety factor}} \times \frac{\text{Molecular weight of cyclamate}}{\text{Molecular weight of CHA}} \times \frac{100\,\%}{\text{Metabolism}}$$

NOEL for CHA

The toxicity of concern in establishing the NOEL is testicular atrophy in rats. Whether this is appropriate has been questioned recently by studies on the metabolism of cyclohexylamine in various species. In the rat, cyclohexylamine undergoes aliphatic hydroxylation to aminocyclohexanols (Renwick and Williams, 1972*a*) (Fig. 3) which are likely to retain sympathomimetic activity, whilst in man cyclohexylamine undergoes deamination. These metabolic pathways represent up to 20 % of the dose in the rat and about 2 % in man. We have shown recently (Roberts & Renwick, 1985) that less than 1 % of a dose undergoes hydroxylation in

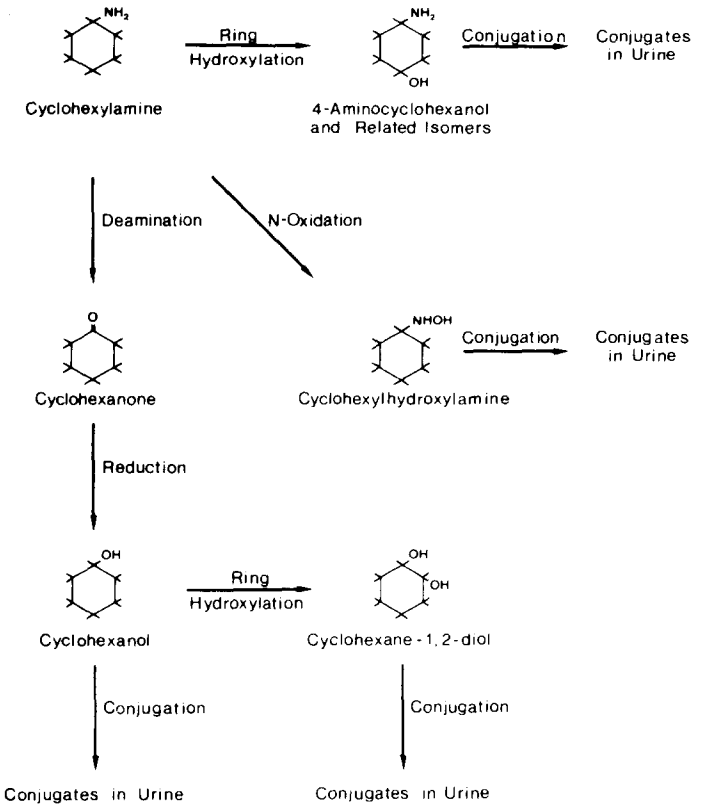

Fig. 3. Pathways of metabolism of cyclohexylamine in animals and man (from Renwick, 1983*a*).

the mouse, a species which appears not to be susceptible to cyclohexylamine-related testicular toxicity. These findings thus raise doubts about the appropriateness of the rat as the species for the prediction of potential risk for man.

Percentage metabolism

In view of the skewed distribution in the extent of metabolism in man (see Fig. 2), the use of the arithmetic mean value (about 2%) would leave a section of the population with a greatly reduced (10–30 fold) safety margin. An alternative would be to use the maximum possible metabolism. Since about 40% of cyclamate is absorbed in the upper intestine and cyclohexylamine formation is a first-pass phenomenon

(Renwick, 1982), only the unabsorbed fraction (60%) will be available for bacterial metabolism and this will be the maximum. However, such an approach would apply a 100-fold safety factor to the 99th percentile of conversion. In view of the extensive human metabolism data (over 200 subjects during chronic intake), this appears to be an over-conservative approach. This conclusion is supported by a study in Japan in 1969 when cyclamate was used extensively as a food additive (Asahina *et al.*, 1971). Twenty-four hour urine samples were collected from 50 people on a 'normal' diet and analysed for cyclohexylamine. The daily excretion was less than 30 mg (0·5 mg kg^{-1}) in 49 of the subjects and 129 mg (2 mg kg^{-1}) in the remaining subject. Thus it is possible that even the high level of use of cyclamate in 1969 would be unlikely to pose a realistic risk.

An alternative theoretical approach is to utilise part of the safety factor (as discussed previously; Renwick, 1983*a*) to incorporate the large amount of human data available. A 100-fold safety factor may be regarded as deriving from 10-fold for the extrapolation from animal to man and 10-fold for inter-individual differences in the human population. The latter factor can be regarded as comprising a 3-fold factor for differences in sensitivity and 3-fold for differences in metabolism. Applying the latter factor to the theoretical maximum conversion (60%) gives a working value of 20% metabolism.

Safety factor

A safety factor of 100 is normally applied to food additives, although this may be either lower or higher depending on the particular toxicological problem. The utilization of this factor in the interpretation of toxicity data is aimed to provide an acceptable level of intake or exposure which could apply to every day of the person's life. However, studies in animals and man (Wills *et al.*, 1981) suggest that a good converter of cyclamate may not retain that activity despite continued intake of cyclamate. In addition, even a brief cessation of cyclamate intake is followed by a loss of metabolic activity in animals and man. Thus the application of a 'lifetime' safety factor in the case of cyclamate would appear over-restrictive.

In conclusion, the use of the equation given is both complex and contentious. An ultra-conservative approach would be to utilize a 100-fold safety factor and 60% metabolism. However, it is unlikely that there would be anyone actually able to metabolize this much cyclamate consistently throughout life. The use of a 20% metabolism value is more

realistic, but would still apply to only 4% of the population during chronic administration, and is not likely to apply to these individuals throughout life.

ASPARTAME

The fate of aspartame in the body is considerably less complex than that of cyclamate. The sweetener is not absorbed as such but is hydrolysed to its constituents, i.e. phenylalanine, aspartic acid and methanol (Fig. 4). Since these are naturally-occurring, and in the case of phenylalanine essential for normal life, the amounts produced from aspartame can be related to a 'natural' experience. As such, this produces an easier and more rational position for regulators since hypothetical risks derived from animal experimentation can be related to endogenous levels of these compounds in the general circulation of man.

Studies in animals have compared the fate of aspartame labelled with

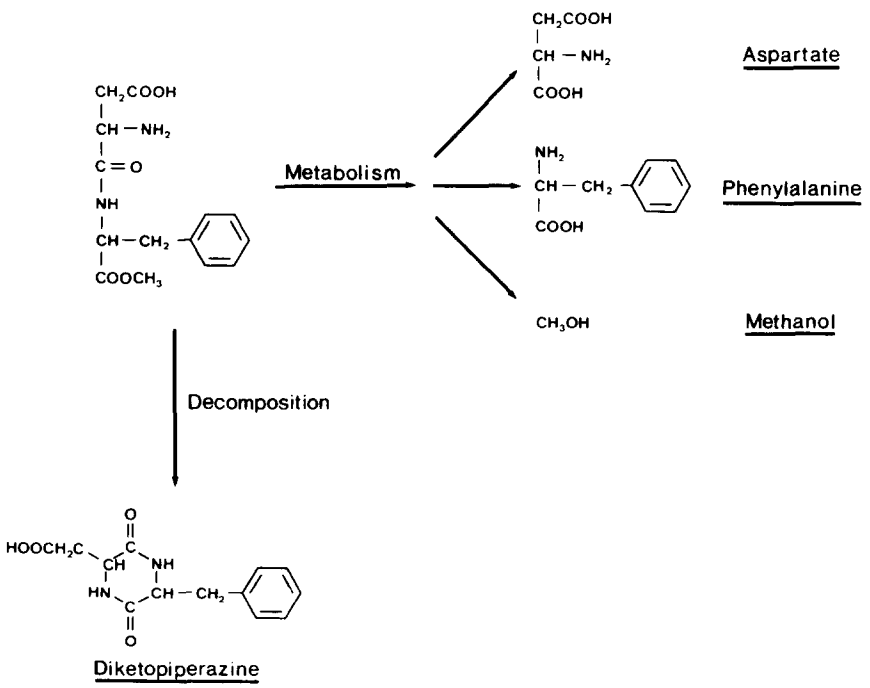

Fig. 4. Pathways of metabolism and decomposition of aspartame.

^{14}C in each of the constituents, with that following the administration of [^{14}C]phenylalanine, [^{14}C]aspartic acid and [^{14}C]methanol (Opperman *et al.*, 1973). Comparison of [^{14}C-phenylalanine]-aspartame with [^{14}C]phenylalanine showed no differences in the plasma ^{14}C–time curves or in the elimination of ^{14}C in urine and faeces. The comparisons of methyl- and aspartyl-labelled aspartame with the radio-labelled constituents gave similar findings, except for the presence of a slower rate of absorption following the labelled aspartame. This is probably due to the time required for the sweetener to be hydrolysed during its absorption from the gut (Heizer & Laster, 1969; Addison *et al.*, 1975).

Studies in man have concentrated on the plasma concentrations of phenylalanine, aspartic acid and methanol following oral administration of aspartame. These studies have included both potentially normal conditions of use and adverse situations, under which it might be anticipated that abnormally high concentrations of the metabolites could be present in the blood. 'High risk' situations which have been studied include:

(i) 'abuse' doses of up to 200 mg kg^{-1}. The replacement of all sucrose in the normal diet would produce an average daily intake of only 8 mg kg^{-1} (Stegink *et al.*, 1979*b*; FDA, 1981);
(ii) various age groups, including one year old infants, given doses of up to 100 mg kg^{-1} (Filer *et al.*, 1983; Stegink *et al.*, 1983);
(iii) lactating women given doses up to 50 mg kg^{-1} (Stegnik *et al.*, 1979*a*);
(iv) subjects heterozygous for phenylketonuria (Stegink *et al.*, 1980) who would be expected to show impaired metabolism of phenylalanine;
(v) in combination with monosodium glutamate (Stegink *et al.*, 1979*b*).

In summary, these various studies have shown that:

(a) there is no significant increase in aspartate in the blood at doses less than 100 mg kg^{-1};
(b) the plasma concentration of phenylalanine increased following aspartame, but for normal subjects remained within the normal post-prandial range after doses of 34 mg kg^{-1}. At higher doses and in phenylketonuria heterozygous individuals, the increase in phenylalanine slightly exceeded the normal post-prandial range;

(c) the amounts of methanol released are toxicologically insignificant;
(d) the increase in phenylalanine in the milk of lactating women was within the range of post-prandial changes. Changes in infants were similar to those in adults;
(e) although a combination of monosodium glutamate plus aspartame, at doses of 34 mg kg^{-1} of each, increased the plasma levels of total glutamate plus aspartate, the increase (about 6 μmol per 100 ml) did not approach the level of 100 μmol per 100 ml necessary to produce symptoms of neurotoxicity in animals.

In conclusion, it appears that the fate of aspartame can be described adequately by that of its constituents. Any toxicological risk can then be put into the context of the risk associated with these constituents. Thus, individuals homozygous for phenylketonuria are warned about the phenylalanine content of the compound. Recent reports that aspartame (at 200 mg kg^{-1}) increases the concentrations of phenylalanine and its metabolite, tyrosine, in the brains of rats (Wurtman, 1983) have received much attention. In addition, it has been shown that the changes in other amino acids which occur normally in response to glucose can be blocked, and that these aspartame-induced neurochemical effects can have physiological consequences (Maher and Wurtman, 1983). These data (at doses of 3 g kg^{-1} glucose with and without 200 mg kg^{-1} of aspartame) have been considered by regulatory bodies in the UK and USA not to provide evidence of potential risk to man at possible levels of use in the human diet (Yellowlees, 1983).

The use of aspartame is associated with the problem of its decomposition to diketopiperazine (5-benzyl-3,6-dioxo-2-piperazine) (see Fig. 4). Thus, information on the toxicity of its breakdown product has been required prior to its approval for use (Ishii *et al.*, 1981; Miller, 1983). The metabolic fate of diketopiperazine has not been reported.

In conclusion, the assessment of the safety of aspartame is related to the extensive data based on the fate of the compound in man and the possible changes in endogenous intermediary metabolites which result from its use. These various studies have been collected into a recent book entitled *Aspartame—Physiology and Biochemistry* (Stegink and Filer, 1983).

ACESULFAM-K

The fate of acesulfam-K has not been published in detail. Reviews presented at international symposia (Arpe, 1978; Mayer, 1983) have

stated that the sweetener is well and rapidly absorbed from the gut, does not accumulate in the tissues and is eliminated in the urine without undergoing metabolism. Thus its fate in the body is similar to that of saccharin. This is possibly related to the fact that both are acidic cyclic sulphonamide derivatives, and a number of structurally related compounds show a similar absence of biotransformation *in vivo* (Renwick, 1983*b*).

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