Vitis **26**, 43—58 (1987)

SCIENTIA VITIS ET VINI

Testing grapes and wines for naturally occurring mutagenic compounds: A Review

by

R. E. SUBDEN [1])

Natürlich vorkommende mutagene Wirkstoffe in Trauben und Weinen: Übersichtsbericht

Zusammenfassung: Mutagene oder karzinogene Nitro- und Phenolverbindungen, Ester und mikrobielle Wirkstoffe wurden in Mosten und Weinen nachgewiesen. Ihre Konzentrationen waren jedoch niedrig und entsprachen den in Fruchtsäften und anderen Gärungsprodukten gefundenen Werten. Vorkommen, dosisabhängiges, physiologisches Verhalten sowie Methoden der Prüfung auf mutagene und karzinogene Eigenschaften werden besprochen.

Key words: berry, must, wine, microbiology, metabolism, phenol, mutation

Introduction

A correlation between the consumption of alcoholic beverages and oesophageal cancer has been established (MCGLASHAN *et al.* 1968; MCGLASHAN 1969; TUYNS 1970, 1979; WARWICK and HARINGTON 1973; WALKER *et al.* 1979; HOEY *et al.* 1981). The risk of oesophageal cancer for drinkers of wine is less than that for spirits (MANDARD *et al.* 1981). A major cause of cancer, and a number of other diseases, is DNA damage (AMES 1979; HIATT *et al.* 1977) some of which is a result of naturally occurring mutagens in our environment. Because most carcinogens are mutagens (AMES and MCCANN 1976; SUGIMURA *et al.* 1976), a series of short term tests (PURCHASE *et al.* 1976; HOLLSTEIN *et al.* 1979; HEINZE and POULSEN 1983) have been devised that provide a rapid economical and sensitive screening procedure for environmental mutagens. The *Salmonella*/microsome mutagenicity assay has been sufficiently refined and standardized to be able to detect 90 % (MCCANN *et al.* 1975) of a list of 174 known carcinogens as mutagens. Using short term and other assays, mutagens have been detected in orange and grapefruit juice (MAZAKI *et al.* 1982), apple juice (SCOTT *et al.* 1977), tea and coffee (NAGAO *et al.* 1979), grape juice (STOLTZ *et al.* 1982), spirits (NAGAO *et al.* 1981; LOQUET *et al.* 1981; SUBDEN *et al.* 1985), tap water (HEARTLEIN *et al.* 1981) and other beverages. However, long term animal tests for carcinogenicity do not always confirm indications from mutagen assays.

When several different wines were screened with the *Salmonella*/microsome mutagen assay (TAMURA *et al.* 1980; STOLTZ *et al.* 1982; SUBDEN *et al.* 1983 a, b) some red (but no white) wines were found to contain a small but significant (DESERRES and SHELBY 1979) level of mutagen.

The present paper reviews the major short term assays and the occurrence, nature, and metabolism of the naturally occurring mutagens and putative carcinogens of grapes and wines.

[1]) Department of Microbiology, University of Guelph, Guelph, Ontario, Canada

Tests for screening mutagens

As there are more than a dozen short term tests for assaying mutagens only those with publications relevant to grape and wine will be described. Readers are referred to a review by HOLLSTEIN *et al.* (1979) for further study.

1. Sister chromatid exchanges

Some mutagens act by breaking chromosomes (clastogens) and in so doing cause DNA damage affecting gene expression. The clastogen assays involve administration of a chromosome stain or radioactive tracer to cultured cells during one replication and removal at the succeeding replication. There is a low level of spontaneous breaking and reannealing between sister chromatids during cell divisions. The stain or radioactive tracer can be used to determine the frequency of spontaneous sister chromatid exchanges. Clastogens increase the frequency of sister chromatid exchanges.

The three most common flavonols in grapes are quercitrin, quercitin and kaempferol (NUZUBIDGE and GULBANI 1964; RIBÉREAU-GAYON 1964 a, b). Both quercitin and kaempferol have been shown to increase the frequency of sister chromatid exchanges in cultured hamster cells (SUGIMURA 1980; CARVER *et al.* 1983).

2. Cell culture assays

Test compounds can be administered to human or other mammalian cells cultured *in vitro* and one can measure the effect of these compounds on the mutation rate or the ability of the compound to transform normally growing cells into those with the growth parameters of cancer cells. Both quercetin and kaempferol have been shown to mutate (MARUTA *et al.* 1979) and transform (UMEZAWA *et al.* 1977; MELTZ and MACGREGOR 1981) human and mouse cells in culture.

3. *Drosophila* mutagen assay

By studying sex frequencies in dose/survivor populations one can calculate a frequency for the induction of sex-linked and other mutations in *Drosophila melanogaster*. Both quercitin and kaempferol have been shown to be mutagenic in *Drosophila* (WATSON 1982).

4. *Salmonella*/microsomal mutagen assay

By far the most widely accepted and standardized short term assay for mutagens is the AMES test which measures the potential of test compounds to reverse or 'back' mutate certain sites on the histidine operon of *Salmonella typhimurium*. Each mutant site was constructed to be sensitive to a specific class or family of mutagen. Strain TA97 has a CCCCCC run (LEVIN *et al.* 1982 a) and TA98 has a GCGCGCGC run near the site of a frameshift mutation (AMES *et al.* 1975). Such nucleotide sequences are particularly sensitive to mutagenesis by test compounds that cause further frameshift mutations (restoring the reading frame) and so increase the reversion frequency. The strains sensitive to base pair substitution mutagens are TA100 which requires a C→T mutation (AMES *et al.* 1975) and TA102 which requires a T→C mutation (LEVIN *et al.* 1982 b) for reversion. Strain TA102 is selective for certain oxidative mutagens and the sensitivity is increased by having the hist G gene carrying the mutant site on a multicopy plasmid (LEVIN *et al.* 1982 b).

To prevent the bacteria from excluding harmful agents or from correcting reverse mutations caused by test compounds, the rfa mutation was introduced, resulting in a

defective lipopolysaccharide protective outer coating, and the uvr B mutation resulting in a defective mutation repair enzyme. Plasmid pKM101 was also introduced as it increased bacterial sensitivity to mutagens by some unknown mechanism.

Activation systems

Promutagens are compounds that are not mutagenic in themselves but become mutagenic due to chemical and metabolic interactions with other foods, the gut microflora, human liver or other tissue enzymes. To simulate natural events, one step in the *Salmonella*/microsome mutagen assay is to combine the test compound with an enzyme extract (the microsomal or S9 fraction) of rats whose hepatic enzymes had been induced by an interperitoneal injection of polychlorinated biphenyls. *Salmonella* tester strains can then be exposed to the compound plus possible metabolites.

STOLTZ *et al.* (1982) compared the mutagenicity of red and white wine, with and without S9 activation. They found that red and white wines have very low mutagen content but the red wines (more than white) contain a promutagen that is S9 activated to form a mutagenic species affecting TA98 more than TA100 indicating a frameshift mutagen. The same study detected significant levels of mutagen in grape juice.

LIN and TAI (1980) found no mutagens in Chinese wines but found very high levels of mutagen if the wine had been treated with nitrate. Nitrates and nitrites have extensive use in Chinese food processing (LIN 1978) and the nitrate treatment was to simulate food combination activations. It was suggested that the mutagen produced was a nitrosamide as it was highly mutagenic with or without S9 activation.

Many naturally occurring promutagens are glycosides that are not readily activated to form aglycones by mammalian systems. Cycasin (from ferns) is not mutagenic nor is it activated by S9 type preparations. Activation occurs in the presence of β-glucosidases (SMITH 1966; MATSUSHIMA *et al.* 1979; MORGAN and HOFFMANN 1983) (Fig. 1).

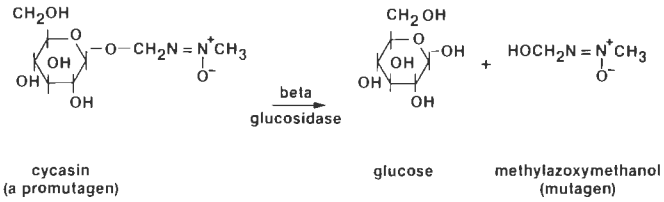

Fig. 1: Cycasin activation.

Cycasinaktivierung.

The gut microflora has an abundance of sugar cleaving enzymes so cycasin is carcinogenic in rats but not in germ-free rats (LAQUER 1964; MATSUMOTO 1980). Several sources of sugar cleaving enzymes have been reported; cecalase from rat feces (BROWN and DIETRICH 1979 a b), hesperidinase from molds (NAGAO *et al.* 1978, 1979) and fecalase from human feces (TAMURA *et al.* 1980).

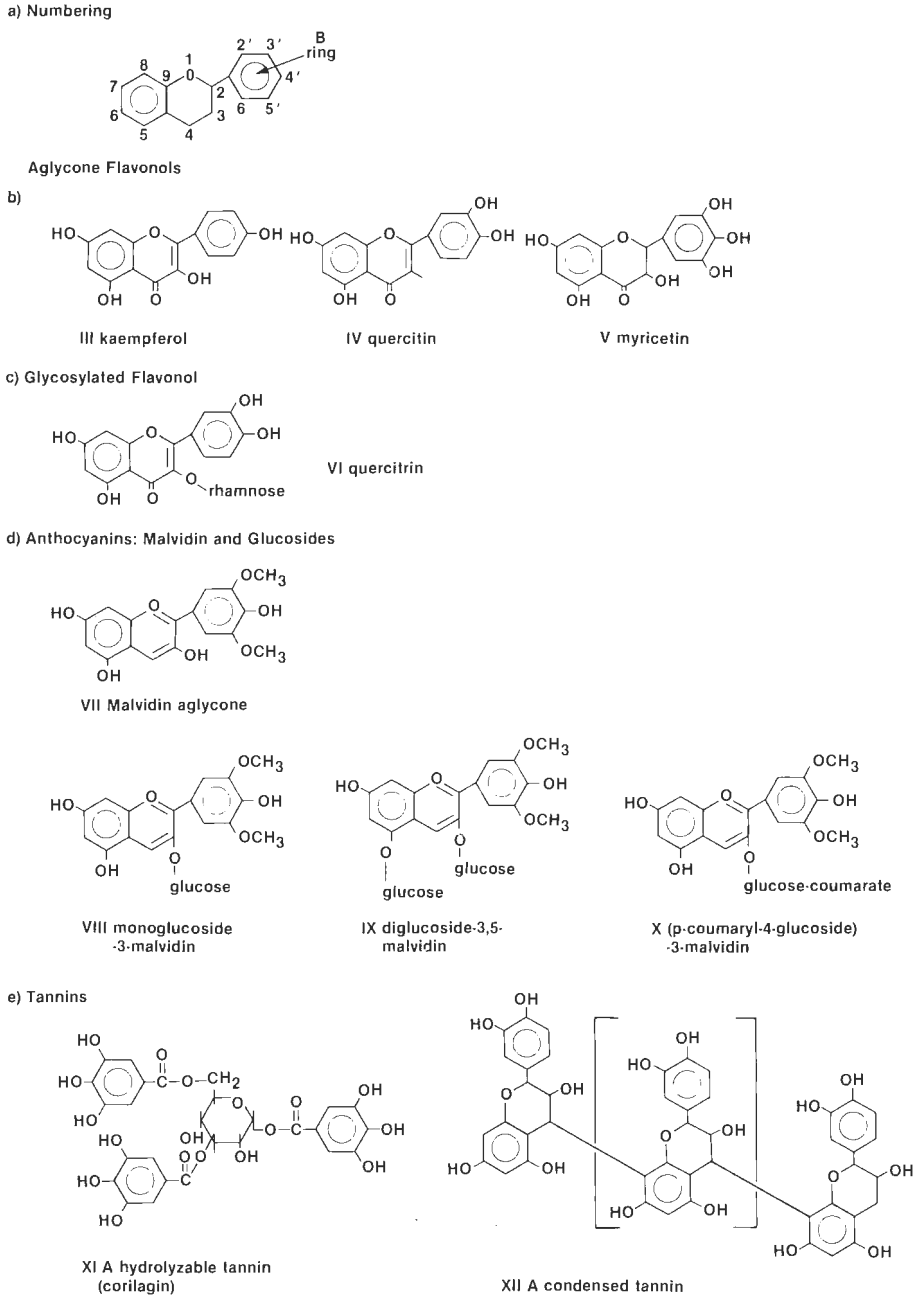

Fig. 2: Some grape polyphenolics.

Polyphenole aus Trauben verschiedener Rebsorten.

TAMURA *et al.* (1980) found that fecalase and S9 activated promutagens in grape juice and red wines but not white. Other reports using fecalase had similar results but the response was also related to grape variety (SUBDEN *et al.* 1983 b) and must processing.

In most countries, grapevine pesticide sprays must demonstrate lack of mutagenicity before they are recommended for use. RASQUINHA *et al.* (1982) produced a grapevine microsomal extract (S14) that activates promutagens including several commonly used grape fungicides and pesticides. It is unlikely, however, that vine activation of spray promutagens is a factor in wine and must mutagenesis.

Promutagen activation in humans is not limited to gut or hepatic metabolism. Activation of several polycyclic hydrocarbons (GUTHRIE *et al.* 1982) and amines (JOSEPHY *et al.* 1981; JOSEPHY and SUBDEN 1984; ROBERTSON *et al.* 1983) is performed by prostaglandin endoperoxide synthetase and other peroxidases. Given the polycyclic nature of some putative grape promutagens it would be of interest to determine their response to peroxidase activation.

Non specific or unknown activations can be estimated from mutagen assays on the urine of subjects which have injested test compounds (RUSSEL and KRAHN 1981). When the urine of wine and grape juice drinkers was screened in the *Salmonella*/microsome assay, no mutagens were detected (SOUSA *et al.* 1985).

Grape and wine mutagens

Although there have been several reports of naturally occurring toxic substances in wines, only those related to mutagens shall be reviewed. Patulin (4-hydroxy-4H-furo[3,2-C]-pyran-2(6H)-one) is a metabolite of certain *Aspergillus, Penicillium* and *Byssochlamys* mold species (SCOTT 1974; ESCOULA 1975). It is a carcinogen (DICKENS and JONES 1961) and has been found on moldy grapes (SOMMER *et al.* 1974). Patulin has been found in grape juice but not in wine (SCOTT *et al.* 1977). Apparently, patulin is unstable in the presence of SO_2 (POHLAND and ALLEN 1970) approximately 47 mg/l of which is produced by yeasts during fermentation (WÜRDIG and SCHLOTTER 1967). Patulin has been reported in apple juice (SCOTT *et al.* 1972) but not in apple cider (HARWIG *et al.* 1973).

The flavonols quercitin and kaempferol are believed to be the principle mutagens in wines primarily through studies with other fruit juices (WOLLENWEBER and DIETZ 1981; MAZAKI *et al.* 1982). Red wines are fermented on the grape skins, from which they extract pigments VII, VIII, IX, X, condensed tannins XII, and flavonols III, IV, V, VI (Fig. 2). White wines are not fermented on the skins and have a lower flavonol content and lower mutagen content (Figs. 3 and 4). Structural biosynthetic relationships and importance of flavonols and other polyphenolics (Fig. 1) have been extensively reviewed elsewhere (SINGLETON and ESSAN 1969; RIBÉREAU-GAYON 1972, 1974; SINGLETON and NOBLE 1976). Condensed tannins XII bind and inactivate proteins such as pectin methylesterases and other hydrolases of fungal pathogens (BACHMANN and BLAICH 1979); flavonoids contribute to oxidative browning (SINGLETON and NOBLE 1976) and tannic acid XI (a contribution from oak cooperage (RIBÉREAU-GAYON 1974)) adds complexity to wine flavors. Activation systems produce mutagenic aglycones (SUGIMURA *et al.* 1977; NAGAO *et al.* 1981) like quercitin IV from promutagens like the rhamnoside quercitrin VI (AMBROSE and ROBBINS 1954). Though structurally similar, few polyphenolics are mutagenic. MACGREGOR and JURD (1978) concluded that the aglycone must have four structural features for mutagenicity viz '(1) a free hydroxyl at the 3 position, (2) a double bond at the 2,3 position (3), a keto group at the 4 position, and (4) a struc-

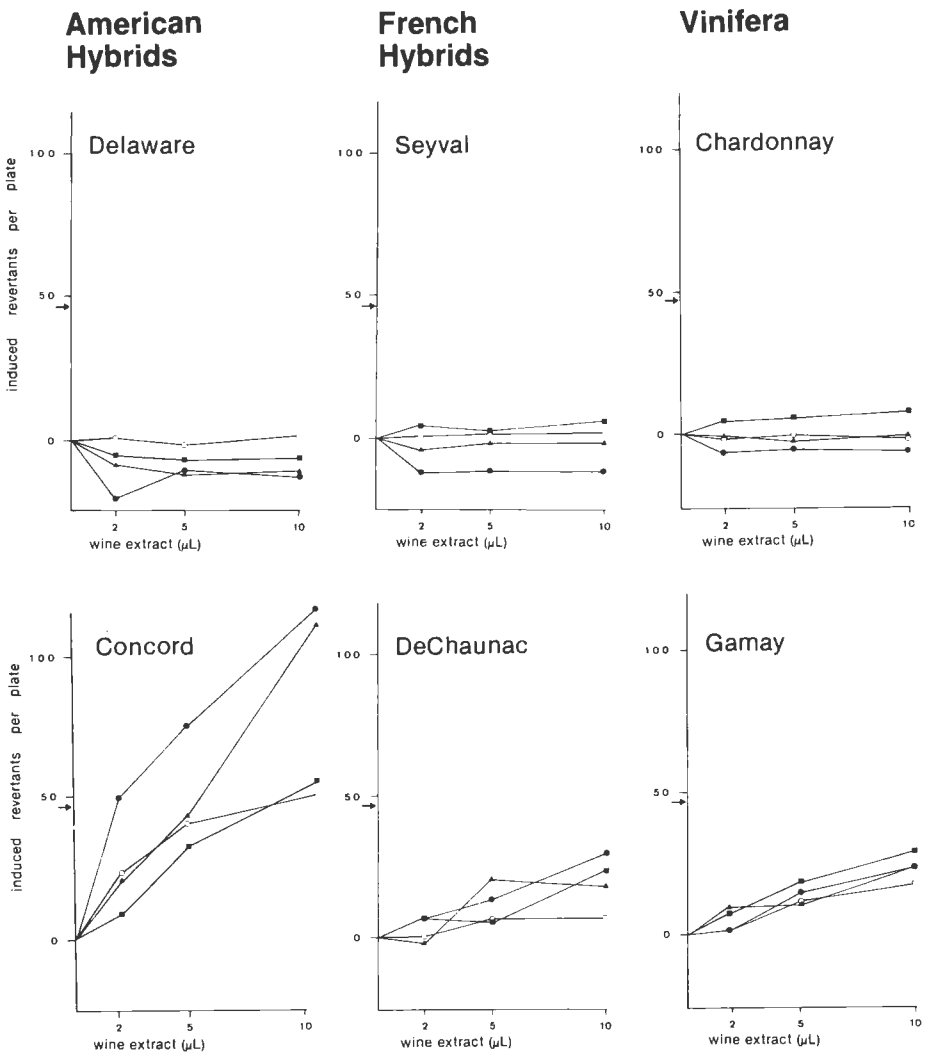

Fig. 3: Mutagen screening using reversion frequencies of *Salmonella typhimurium* strain TA98 exposed to several doses of an Amberlite XAD-2 (nonionic polymeric adsorbant) resin bound extract of wine for various grape species. Frequencies shown have been corrected for spontaneous, nutritional and other effects. The arrow on the ordinate indicates the reversion frequency of statistical significance. — ○ = extract (1 µl extract = 0.25 ml wine); ▲ = extract plus S9 activation; ■ = extract plus fecalase activation; ● = extract plus S9 and fecalase activation.

Bestimmung mutagener Wirkstoffe durch Messung der Rückbildungshäufigkeit von *Salmonella typhimurium* TA98 auf Weinextrakten verschiedener Rebsorten. Die Extrakte wurden mit unterschiedlichen Dosierungen von nicht ionisiertem Amberlite XAD-2-Polymer gewonnen. Zufallsfaktoren in Nährlösungen und Wachstumsbedingungen wurden bereinigt. Der Pfeil auf der Ordinate zeigt statistisch signifikante Häufigkeit. — ○ = Meßwert von 1 µl Extrakt (aus 0,25 ml Wein); ▲ = Extrakt mit S9-Aktivierung; ■ = Extrakt mit Fecalase-Aktivierung; ● = Extrakt mit S9- und Fecalase-Aktivierung.

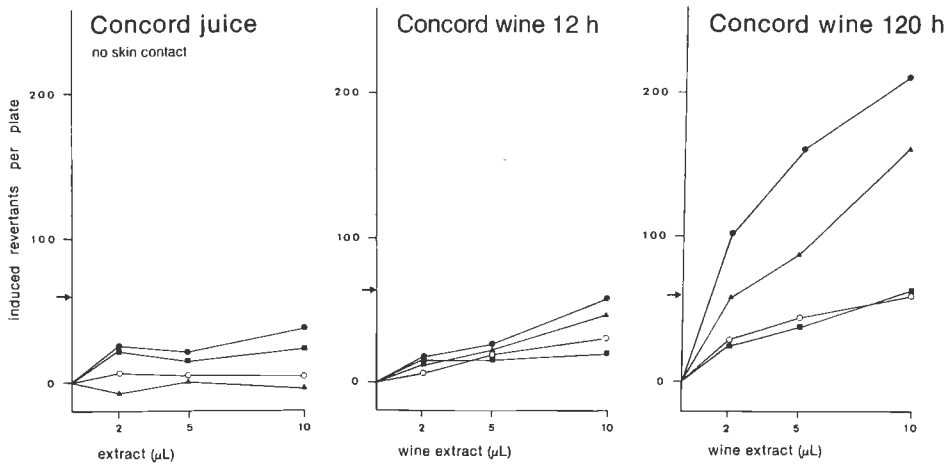

Fig. 4: Mutagen screening using reversion frequencies of *Salmonella typhimurium* strain TA98 exposed to several doses of an Amberlite XAD-2 (nonionic polymeric adsorbant) resin bound extract of Concord wine showing the effects of grape skin contact time during fermentation. Frequencies shown have been corrected for spontaneous, nutritional and other effects. The arrow on the ordinate indicates the reversion frequency of statistical significance. Times shown indicate skin contact time during fermentation. — For further explanation see Fig. 3.

Bestimmung mutagener Wirkstoffe durch Messung der Rückbildungshäufigkeit von *Salmonella typhimurium* TA98 auf Weinextrakten der Concord-Rebe nach Vergärung auf der Maische. Die Extrakte wurden mit dem nicht ionisierten XAD-Polymer gewonnen. Zufallsfaktoren in Nährlösung und Wachstumsbedingungen wurden bereinigt. Der Pfeil auf der Ordinate zeigt statistisch signifikante Häufigkeit. Die angegebenen Zeiten bedeuten die Dauer der Maischegärung. — Weitere Erläuterungen s. Fig. 3.

ture which permits the proton of the 3-hydroxyl group to tautomerize to a 3-keto compound.' Anthocyanins and tannins are not strong mutagens and would require extensive activation to become mutagens according to MACGREGOR and JURD's (1978) criteria.

American *Vitis* species have more widely distributed 3,5 diglucoside anthocyanin pigments IX than *V. vinifera* which has primarily 3-mono glucoside anthocyanins VIII (RIBÉREAU-GAYON 1974; AMERINE *et al.* 1980). Whether there are analogous 3-mono and 3,5 diglucoside flavonoids (which would be promutagens) is not completely resolved (LEA *et al.* 1979), so the chemical bases for species differences in mutagen content (SUBDEN *et al.* 1983 b) are not known.

It would be of some interest to determine if the lactic bacteria that perform malolactic fermentations have β-glucosidases and other flavonol activating or degrading enzymes. The effect of aging, which tends to polymerize polyphenolics, on the mutagen content of wine has not been reported.

Yeast mutagens

Wild yeasts that commonly contaminate wines and produce off odours, flavours and other spoilage effects were collected and identified (SUBDEN *et al.* 1985). The various spoilage yeast species were then used in a *Salmonella*/microsomal mutagen assay

to determine if any of the metabolites were mutagenic. No mutagens were detected in any of the wines made with the contaminating or control (*Saccharomyces cerevisiae* var. Montrachet and *S. beticus*) wine yeasts.

OBE *et al.* (1980) noted that alcoholics have a higher than normal number of lymphocytes with chromosomal aberrations. Further studies indicated that an increase in sister chromatid exchanges could be detected in cultured cells exposed to fusel oil congeners (HOEFT and OBE 1983), acetaldehyde (OBE 1981; OBE *et al.* 1977), and indirectly to ethanol (OBE and RISTOW 1979).

A common metabolite of both yeast and bacterial fermentations is ethyl carbamate or 'urethane'. Concern over the incidence of urethane in wines resulted in the removal of several wines from provincial retail wine stores in Canada in 1985. Urethane had been used as an anesthetic until it was noted (NETTLESHIP *et al.* 1943) that it rapidly induced lung adenomas in mice. Since that time, a number of studies have confirmed the tumorigenic and carcinogenic nature of urethane (MIRVISH 1968; INNES *et al.* 1969; TOTH and BOREISHA 1969; Int. Agency for Res. on Cancer, 1974; World Health Organization, 1972; SCHMAEL *et al.* 1977). Urethane exhibits limited mutagenic activity in the AMES test although it is a carcinogen (DAHL *et al.* 1978).

Rodent tests revealed that 90 % of ^{14}C-carboxy and ^{14}C-methylene-urethane ($NH_2 \cdot {}^{14}CO \cdot OCH_2CH_3$ and $NH_2 \cdot CO \cdot O{}^{14}CH_2CH_3$) is degraded and expired as $^{14}CO_2$ within 24 h while the remainder is voided in the urine or (5 %) resident in body fluids (SKIPPER *et al.* 1951). Some of the urethane may be catabolized by a minor pathway to produce degradary intermediates that are more carcinogenic than urethane (DAHL *et al.* 1978). One study on Californian wines found that less than 6 µg urethane per litre of wine is produced through natural fermentations (OOUGH 1976 a). The same study suggested that the source of natural urethane was esterification of ethanol and carbamyl phosphate:

$$NH_2 \cdot CO \cdot O \cdot PO_3H_2 + CH_3CH_2OH \rightarrow CH_3CH_2 \cdot O \cdot CO \cdot NH_2 + H_3PO_4.$$

Diethyl dicarbonate (DEPC) is a potent antimicrobial agent and has been used extensively in the soft drink industry. In 1972, the World Health Organization (WHO) set 10 µg DEPC/l of soft drink as a permissible level (World Health Organization 1972). The WHO report was careful to specify that DEPC should only be used for beverages with a pH less than 4 and should not be used for high ammonia or amine, containing beverages such as milk. The reason for this is that DEPC can act with primary amines to form urethane:

$$CH_3CH_2 \cdot O \cdot CO \cdot O \cdot CO \cdot O \cdot CH_2CH_3 + RNH_2 \rightarrow 2\,CH_3CH_2 \cdot O \cdot CO \cdot NHR + ETOH + CO_2.$$

There is a report that white wine containing 5 mg/l ammonia dosed with 500 mg/l DEPC produced 2600 µg urethane/l using isotope dilution analysis (LOEFROTH and GEJVALL 1971). Other groups using GLC/MS analysis have disputed these values (FISCHER 1972; OUGH 1976 b; U.S. Dept. of Health Educ. and Welfare 1972) and claimed that they produced levels 100—200 times less than the initial report. Notwithstanding the disputed analysis, the U.S. Federal Food Drug and Cosmetic Act was ammended to rescind permission to use DEPC in wines (Fed. Regist, 1972).

The Liquor Control Board of Ontario (Canada) laboratories used a capillary GLC equipped with a Hall N detector to analyze a wide variety of wines and found concentrations as high as 2500 µg urethane/l of wines, fruit wines, sake and distillates from a number of countries. The occurrence of high levels of urethane has been tentatively associated with the use of urea as a yeast nutrient for low N musts:

$$NH_2C \cdot O \cdot NH_2 + CH_3CH_2OH \rightarrow CH_3CH_2O \cdot CO \cdot NH_2 + NH_3.$$

The Health Protection Branch of the Canadian Federal Government has set 30 µg urethane/l as the permissible limit. At present, the limit is under study as there is no

universally accepted reliable analytic procedure for measuring urethane concentrations at the μg/l level.

Conclusions

It has been adequately demonstrated that (1) red wine has a constituent that is mutagenic in the *Salmonella*/microsome mutagen assay, (2) wine contains quercitin proportional to its mutagenic potential, and (3) quercitin is mutagenic. The etiology of cancer and red wine consumption is vastly different from that of smoking and lung cancer. One can accurately time plot the onset of smoking in the population, the 20 year latency period for cancer incubation and the onset of lung cancers 20 years later with a parallel incidence to that for smoking. Humans had not evolved the catabolic capacity in the respiratory tract to cope with smoke constituents. The average western diet includes about one gram of flavonoids per day (BROWN 1980) 50 mg of which can be classed as mutagen or promutagen. Quercitin has been found in grapefruit and orange juice (MAZAKI *et al.* 1982) tea (NAGAO *et al.* 1979), grape juice (STOLTZ *et al.* 1982) and various other foodstuffs and in each case it was found to be mutagenic in the *Salmonella*/microsome mutagen assay. How can one resolve the presence of a mutagen in significant quantities, widely distributed in human diets and the lack of etiological correlations with specific cancers?

Oral doses of quercitin are not absorbed into the bloodstream (GUGLER *et al.* 1975) and there is no evidence that alcohol potentiates uptake. The ability of the gut microflora to degrade flavones is quite considerable. Only half of a large (70 mg/kg body wt) orally adminstered dose of quercitin is recovered from the feces, and hesperetin, a related flavone, is completely degraded by the gut microflora (HONOHAN *et al.* 1976). One would expect therefore that the effects of quercitin would be greater in the upper parts of the gastrointestinal tract. Flavonoids are not found in spirits and were not the causative agents for oesophageal cancers described by TUYNS *et al.* 1980).

Mice (SUGIMURA 1980; SAITO *et al.* 1980) fed high quercitin diets over a long period of time had tumor and cancer frequencies not different from the control populations. Similar results were reported with rats (HIRONO *et al.* 1981) although there is one reported positive correlation (PAMACKU *et al.* 1980). These data would suggest that though quercitin is mutagenic in the *Salmonella*/microsome and other short term mutagen assays it is probably not a carcinogen in mammals, though certain caution must be exercised with experiments involving large doses given to a few inbred laboratory animals fed a restricted diet over many generations (STREISINGER 1983).

It would seem therefore that mammals have some physiological barrier or tolerance in the upper gastrointestinal tract preventing quercitin access to the DNA. As yet, the nature and conditions optimizing the activity of this barrier have not been elucidated.

Ethyl carbamate or urethane in animal tests is a proven carcinogen and tumorigen when administered at concentrations measured in grams or mg urethane per kilogram body weight. In order to assess risks from exposure to naturally occurring concentrations of urethane at the ng urethane per kilogram body weight, one must calculate the effect through regression analysis and extrapolation of dose/response curves derived from higher dose concentrations of urethane. When such calculations are made, it can be shown that the effect of naturally occurring urethane in wines is considerably less than the standard error (variation) in the spontaneous incidence of tumors and carcinomas.

Summary

Some phenolics, esters, nitro-compounds and mold metabolites that have been shown to be mutagenic or carcinogenic have been detected in musts and wines. The low concentrations of these compounds in musts and wines are similar to those found in other fruit juices and fermented products. The occurrence, physiology, dose-response significance and methods for mutagenicity and carcinogenicity screening of musts and wines are discussed.

Acknowledgements

Financial support of Health and Welfare Canada is acknowledged. Assistance of T. HAFFIE, D. STOLTZ, D. RANCOURT, S. RANCOURT, A. KRIZUS and D. JOSEPHY is greatly appreciated.

References

AMBROSE, A. M.; ROBBINS, D. J.; 1954: Comparative toxicities of quercitin and quercitrin. J. Amer. Pharm. Assoc. Sci. **16**, 119—122.

AMERINE, M. A.; BERG, H. W.; KUNKEE, R. E.; OUGH, C. S.; SINGLETON, V. L.; WEBB, A. D.; 1980: In: The Technology of Wine Making. AVI Publishing Co., Westport, CT., U. S. A.

AMES, B. N.; 1979: Identifying environmental chemicals causing mutations and cancer. Science **204**, 587—593.

— — ; MCCANN, J.; 1976: Carcinogens are mutagens: A simple test system. Int. Agency Res. Cancer Sci. Publ. **12**, 493—504.

— — ; — —; YAMASAKI, E.; 1975: Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat. Res. **31**, 347—364.

BACHMANN, O.; BLAICH, R.; 1979: Vorkommen und Eigenschaften kondensierter Tannine in Vitaceen. Vitis **18**, 106—116.

BROWN, J. P.; 1980: A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat. Res. **75**, 243—277.

— — ; DIETRICH, P. S.; 1979 a: Mutagenicity of plant flavonol glycosides by mixed glycosidase from rat cecal bacteria and other sources. Mutat. Res. **66**, 233—240.

— — ; — —; 1979 b: Mutagenicity of anthraquinone and benzanthrone derivatives in the *Salmonella*/microsome test: activation of anthraquinone glycosides by enzymic extracts of rat cecal bacteria. Mutat. Res. **66**, 9—24.

CARVER, J. H.; CARRANO, A. V.; MACGREGOR, J. T.; 1983: Genetic effects of flavonols quercitin, kaempferol and galagin on Chinese hamster ovary cells *in vitro*. Mutat. Res. **113**, 45—60.

DAHL, G. A.; MILLER, J. A.; MILLER, E. C.; 1978: Vinyl carbamate as a promutagen and a more carcinogenic analog of ethyl carbamate. Cancer Res. **38**, 3793—3804.

Department of Health, Education and Welfare, U. S. A.; 1972: 'Studies on the formation of ethyl carbamate as a reaction production of diethyl dicarbonate and ammonium ion in acidic beverages'. In the Final Reports Sections C10, C32 C33, C35, submitted to Bureau of Foods, Washington, D. C., May 4, 1972.

DESERRES, F. J.; SHELBY, M. D.; 1979: Recommendations on data production and analysis using the *Salmonella*/microsome mutagenicity assay. Environ. Mutagen **1**, 87—92.

DICKENS, F.; JONES, H. E. H.; 1961: Carcinogenic activity of a series of reactive lactones and related substances. Br. J. Cancer **15** (1), 85—100.

ESCOULA, L.; 1975: Moissisures toxinogènes des fourrages ensiles. Ann. Rech. Vet. **6** (3), 303—310.

FISCHER, E.; 1972: Über die Bildung von Carbaminsäureäthylester (Urethan) in Getränken nach Behandlung mit Pyrokohlensäurediäthylester. Z. Lebensm.-Untersuch. Forsch. **221**, 147—148.

GUGLER, R.; LESCHIK, M.; DENGLER, H. J.; 1975: Disposition of quercitin in man after single oral and intravenous doses. Eur. J. Clin. Pharmacol. **9**, 229—234.

GUTHRIE, J.; ROBERTSON, I. G. C.; ZEIGER, E.; BOYD, J. A.; ELING, T. E.; 1982: Selective activation of some dihydrodiols of several polycyclic aromatic hydrocarbons to mutagenic products by prostaglandin synthetase. Cancer Res. **42**, 1620—1623.

HARWIG, J.; SCOTT, P. M.; KENNEDY, B. P. C.; CHEN, Y. K.; 1973: Disappearance of patulin from apple juice fermented by *Saccharomyces* spp. Can. Inst. Fd. Sci. Tech. J. **6** (1), 45—50.

HEARTLEIN, M. W.; DEMARINI, D. M.; KATZ, A. J.; MEANS, J. C.; PLEWA, M. J.; BROCKMAN, H. E.; 1981: Mutagenicity of municipal water obtained from an agricultural area. Environ. Mutagen. **3**, 519—530.

HEINZE, J. E.; POULSEN, N. K.; 1983: The optimal design of batteries of short-term tests for detecting carcinogens. Mutat. Res. **117**, 259—269.

HIATT, H. H.; WATSON, J. D.; WINSTEN, J. A. (Eds.); 1977: Origins of Human Cancer. Cold Spring Harbor Lab., Cold Spring Harbor, New York.

HIRONO, I.; UENO, I.; HOSAKA, S.; TAKANASHI, H.; MATSUSHIMA, T.; SUGIMURA, T.; NATORI, S.; 1981: Carcinogenicity examination of quercitin and rutin in ACI rats. Cancer Lett. **13**, 15—21.

HOEFT, H.; OBE, G.; 1983: SCE inducing congeners in alcoholic beverages. Mutat. Res. **121**, 247—251.

HOEY, J.; MONTVERNAY, C.; LAMBERT, 1981: Wine and tobacco: risk factors for gastric cancer in France. Amer. J. Epidemiol. **113**, 668—674.

HOLLSTEIN, M.; MCCANN, J.; ANGELOSANTO, F. A.; NICHOLS, W. W.; 1979: Short term tests for carcinogens and mutagens. Mutat. Res. **65**, 133—226.

HONOHAN, T.; HALE, R. L.; BROWN, J. P.; WINGARD, R. W.; 1976: Synthesis and metabolic fate of hesperetin-3-^{14}C. J. Agricult. Food Chem. **24**, 906—911.

INNES, J. R. M.; ULLAND, B. M.; VALERIO, M. G.; PETRUCELLI, L.; FISHBEIN, L.; HART, E. R.; PALLOTTA, A. J.; BATES, R. R.; FALK, H. L.; GART, J. J.; KLEIN, M.; MITCHELL, I.; PETERS, J.; 1969: Bioassay of pesticides and industrial chemicals for tumorigencity in mice: A preliminary note. J. Nat. Cancer Inst. **42**, 1101—1114.

International Agency for Research on Cancer; 1974: 'Urethan' in IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Vol. **7**, pp. 111—140. IARC, Lyons.

JOSEPHY, P. D.; MASON, R. P.; ELING, T.; 1981: Cooxidation of the clinical reagent 3,5,3'5'-tetramethylbenzidine by prostaglandin synthase. Cancer Res. **42**, 2567—2570.

— — ; SUBDEN, R. E.; 1984: Hydrogen peroxide-dependent activation of benzidine to mutagenic species. Mutat. Res. **141**, 23—28.

LAQUER, G. L.; 1964: Carcinogenic effects of cycad meal and cycasin methylazoxymethanol glycoside in rats and the effects of cycasin germ free rats. Fed. Proc. **23**, 1386—1388.

LEA, A. G. H.; BRIDLE, P.; TIMBERLAKE, C. F.; SINGLETON, V. L.; 1979: The procyanidins of white grapes and wines. Amer. J. Enol. Viticult. **30**, 289—300.

LEVIN, D. E.; YAMASAKI, E.; AMES, B. N.; 1982 a: A new *Salmonella* tester strain TA97 for the detection of frameshift mutagens. A run of cytosines as a mutational hot-spot. Mutat. Res. **94**, 315—320.

— — ; HOLLSTEIN, M.; CHRISTMAN, M. F.; SCHWIERS, E. A.; AMES, B. N.; 1982 b: A new *Salmonella* tester strain TA102 with A-T pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. U. S. A. **79**, 7445—7449.

LIN, J. K.; 1978: Studies on the levels and toxicities of nitrate and nitrosamine in Chinese food. Nat. Sci. Council Monthly (ROC) **6**, 155—160.

LIN, J. Y.; TAI, M. W.; 1980: Mutagenicity of Chinese wine treated with nitrate. Fd. Cosmet. Toxicol. **18**, 241—243.

LOEFROTH, G.; GEIJVALL, T.; 1971: Diethyl pyrocarbonate: Formation of urethane in treated beverages. Science **174**, 1248—1250.

LOQUET, C.; TOUSSAINT, G.; LETALAER, J. Y.; 1981: Studies on mutagenic constituents of apple brandy and various alcoholic beverages collected in western France, a high incidence area for oesophageal cancer. Mutat. Res. **88**, 155—164.

MACGREGOR, J. T.; JURD, L.; 1978: Mutagenicity of plant flavonoids: Structural requirements for mutagenic activity in *Salmonella typhimurium*. Mutat. Res. **54**, 297—309.

MANDARD, A. M.; MARNAY, J.; HELIE, H.; TUYNS, A. I.; LETALAER, I. Y.; 1981: Recherches concernant les facteurs étiologiques du cancer de l'oesophage dans l'ouest de la France. Bull. Cancer **68** (1), 49—58.

MARUTA, A.; ENAKA, K.; UMEDA, M.; 1979: Mutagenicity of quercetin and kaempferol on cultured mammalian cells. Gann **70**, 273—276.

MATSUMOTO, H.; 1980: In: MILLER E. C. *et al.* (Eds): Naturally Occurring Carcinogens — Mutagens and Modulators of Carcinogenesis. Japan. Sci. Soc. Press, Tokyo, pp. 67—77.

MATSUSHIMA, T.; MATSUMOTO, H.; SHIRAI, A.; SAWAMURA, M.; SUGIMURA, T.; 1979: Mutagenicity of the naturally occurring carcinogen cycasin and synthetic methylazoxymethanol conjugates in *Salmonella typhimurium*. Cancer Res. **39**, 3780—3782.

MAZAKI, M.; ISHII, T.; UYETA, M.; 1982: Mutagenicity of hydrolysates of citrus fruit juices. Mutat. Res. **101**, 283—291.

MCCANN, J.; CHOI, E.; YAMASAKI, E.; AMES, B. N.; 1975: Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. U. S. A. **72**, 5135—5139.

McGLASHAN, N. D.; 1969: Oesophageal cancer and alcoholic spirits in Central Africa. Gut **10**, 643—647.

— — ; WALTERS, C. L.; McLEAN, A. E. M.; 1968: Nitrosamines in African alcoholic spirits and oesophageal cancer. Lancet **II**, 1017—1021.

MELTZ, M. L.; MACGREGOR, J. T.; 1981: Activity of plant flavonol quercitin in the mouse lymphoma L5178YTK +/− mutation. DNA single strand break and Balb/c 3T3 chemical transformation assays. Mutat. Res. **88**, 317—324.

MIRVISH, S. S.; 1968: The carcinogenic action and metabolism of urethan and N-hydroxyurethan. Adv. Cancer Res. **11**, 1—42.

MORGAN, R. W.; HOFFMANN, G. R.; 1983: Cycasin and its mutagenic metabolites. Mutat. Res. **114**, 19—58.

NAGAO, M.; MORITA, N.; YAHAGI T.; SHIMIZU, M.; KUROYANAGI, M.; FUKUOKA, M.; YOSHIHIRA, K.; NATORI, S.; FUNJANO, T.; SUGIMURA, T.; 1981: Mutagenicities of 61 flavonoids and 11 related compounds. Environ. Mutagen **3**, 401—419.

— — ; TAKAHASHI, Y.; WAKABAYASHI, K.; SUGIMURA, T.; 1981: Mutagenicity of alcoholic beverages. Mutat. Res. **88**, 147—154.

— — ; — —; YAMANAKA, H.; SUGIMURA, T.; 1979: Mutagens in coffee and tea. Mutat. Res. **68**, 101—106.

NAGAO, N.; SUGIMURA, T.; MATSUSHIMA, T.; 1978: Environmental mutagens and carcinogens. Ann. Rev. Genetics **12**, 117—159.

NETTLESHIP, A.; HENSHAW, P. S.; MEYER, H. L.; 1943: Induction of pulmonary tumors in mice with ethyl carbamate (urethane). J. Nat. Cancer Inst. **4**, 309—315.

NUZUBIDGE, N. N.; GULBANI, D. J.; 1964: Flavonols in grapes (transl.). Soobshcheniya Akad. Nauk Gruz. SSR **36**, 345—352.

OBE, G.; 1981: Acetaldehyde not ethanol is mutagenic. In: KAPPAS, A. (Ed.): Progress in Mutation Research. Elsevier/North Holland Press, Amsterdam, pp. 19—23.

— — ; GOEBEL, D.; ENGELN, H.; HERHA, J.; NATARAJAN, A. T.; 1980: Chromosomal aberrations in peripheral lymphocytes of alcoholics. Mutat. Res. **73**, 377—386.

— — ; RISTOW, H.; 1977: Acetaldehyde but not ethanol induces sister chromatid exchange in Chinese hamster cells *in vitro*. Mutat. Res. **56**, 211—213.

— — ; — —; 1979: Mutagenic carcinogenic, teratogenic effects of alcohol. Mutat. Res. **65**, 229—259.

OUGH, C. S.; 1976 a: Ethylcarbamate in fermented beverages and foods. I. Naturally occurring ethyl carbamate. J. Agricult. Food Chem. **24**, 323—328.

— — ; 1976 b: Ethylcarbamate in fermented beverages and foods. II. Possible formation of ethylcarbamate from diethyl dicarbonate addition to wine. J. Agricult. Food Chem. **24**, 328—331.

PAMACKU, A. M.; YALCINER, S.; HATCHER, J.; BRYAN, G. T.; 1980: Quercitin: A rat intestinal and bladder carcinogen present in braken fern (*Pteridium aquilinum*). Cancer Res. **40**, 3468—3472.

POHLAND, A. E.; ALLEN, R. J.; 1970: Stability studies with patulin. J. Assoc. Offic. Analyt. Chem. **53** (4), 688—692.

PURCHASE, I. F. M.; LONGSTAFF, E.; ASHBY, J. A.; ANDERSON, D.; LEFEVRE, P. A.; WESTWOOD, F. R.; 1976: Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use. Nature (London) **264**, 624—627.

RASQUINHA, I.; WILDEMAN, A.; NAZAR, R. N.; SUBDEN, R. E.; 1982: A method for determining the effects of grapevine metabolism on the mutagenicity of fungicides and pesticides. Amer. J. Enol. Viticult. **33**, 178—180.

RIBÉREAU-GAYON, P.; 1964 a: Les composés phénoliques du raisin et du vin. Inst. Natl. de la Rech. Agron. Paris, 83 pp.

— — ; 1964 b: Les composés phénoliques du raisin et du vin. I. II. III. Ann. Physiol. Vég. **6**, 119—147, 211—242, 259—282.

— — ; 1972: In: Plant Phenolics. Oliver and Boyd Pub., Edinburgh, U. K.

— — ; 1974: The chemistry of red wine colour. In: Chemistry of Winemaking. Amer. Chem. Soc., Washington, D. C., pp. 50—87.

ROBERTSON, I. G. C.; SIVARAJAH, K.; ELING, T. E.; ZEIGER, E.; 1983: Activation of some aromatic amines to mutagenic products by prostaglandin endoperoxide synthetase. Cancer Res. **43**, 476—480.

RUSSELL, J. F.; KRAHN, O. F.; 1981: Development of protocol for evaluating the mutagenic activity of human urine in the *Salmonella typhimurium* suspension assay. Environ. Mutagen **6**, 476—478.

SAITO, D.; SHIRAI, A.; MATSUSHIMA, T.; SUGIMURA, T.; HIRONO, I.; 1980: Test of carcinogenicity of quercitin a widely distributed mutagen in food. Teratogen. Carcinogen. Mutagen. **1**, 213—221.

SCHMAEL, D.; PORT, R.; WAHRENDARF, J.; 1977: A dose-response study on urethane carcinogenesis in rats and mice. Int. J. Cancer **19**, 77—80.

SCOTT, P. M.; 1974: In: PURCHASE, I. F. H. (Ed.): Mycotoxins. Elsevier Press, Amsterdam, pp. 383—403.

— — ; MILES, W. F.; TOFT, P.; DUBE, J. G.; 1972: Occurrence of patulin in apple juice. J. Agricult. Food Chem. **20**, 450—451.

— — ; FULEKI, T.; HARWIG, J.; 1977: Patulin content of juice and wine produced from moldy grapes. J. Agricult. Food Chem. **25**, 434—437.

SINGLETON, V. L.; ESSAN, P.; 1969: Phenolic substances in grapes and wines and their significance. Adv. Food Res. Supp. 1, Academic Press, N. Y.

— — ; NOBLE, A. C.; 1976: Wine flavour and phenolic substances. In: CHARALAMBOUS, G.; KATZ, I. (Eds.): Phenolic, Sulfur and Nitrogen Compounds in Food Flavours. Symp. Series No. 26, pp. 47—70. Amer. Chem. Soc. Washington, D. C.

SKIPPER, H. E.; BENNETT, L. L.; BRYAN, C. E.; WHITE, L.; NEWTON, M. A.; SAMPSON, L.; 1951: Carbamates in the chemotherapy of leukemia. VIII. Overall trace studies on carbonyl-labelled urethane, methylene-labelled urethane, and methylene-labelled alcohol. Cancer Res. **11**, 46—51.

SMITH, D. W. E.; 1966: Mutagenicity of cycasin aglycone (methylazoxymethanol) a naturally occurring carcinogen. Science **152**, 1273—1274.

SOMMER, N. F.; BUCHANAN, J. R.; FORTLAGE, R. J.; 1974: Production of patulin by *Penicillium expansum*. Appl. Microbiol. **28**, 589—592.

SOUSA, J.; NATH, J.; ONG, T.; 1985: Dietary factor affecting the urinary mutagenicity assay system. II. The absence of mutagenic activity in human urine following consumption of red wine or grape juice. Mutat. Res. **156**, 171—176.

STOLTZ, D. R.; STAVRIC, B.; KREWSKI, D.; KLASSEN, R.; BENDALL, R.; JUNKINS, B.; 1982: Mutagenicity screening of foods. I. Results with beverages. Environ. Mutagen. **4**, 477—492.

STREISINGER, G.; 1983: Extrapolations from species to species and from various cell types in assessing risks from chemical mutagens. Mutat. Res. **114**, 93—105.

SUBDEN, R. E.; HAFFIE, T.; RANCOURT, D.; 1983 a: The mutagen content of homemade Canadian wines. Can. Inst. Food Sci. Technol. J. **16**, 45—51.

— — ; KRIZUS, A.; AKHTAR, M.; 1986: Mutagen content of Canadian apple eaux-de-vie. Can. Inst. Food Sci. Technol. J. **19**, 134—136.

— — ; — —; JOSEPHY, D.; 1985: Mutagen content of wines contaminated with common yeasts. Food Chem. Toxic. **23**, 343—347.

— — ; — —; RANCOURT, D.; 1983 b: Mutagen content of table wines made from various grape species and hybrid cultivars. Food Chem. Toxic. **22**, 309—313.

SUGIMURA, T.; 1980: In: MILLER, E. C. *et al.* (Eds.): Naturally Occurring Carcinogens. — Mutagens and Modulators of Carcinogenesis. Japan. Sci. Soc. Press, Tokyo. University Press, Baltimore, pp. 241—261.

— — ; NAGAO, M.; MATSUSHIMA, T.; YAHAGI, T.; SEINO, Y.; SHIRAI, A.; SAWAMURA, M.; NATORI, S.; YOSHIHARA, K.; FUKUOKA, M.; KUROYANGI, M.; 1977: Mutagenicity of flavone derivatives. Proc. Japan. Acad. **53 B**, 194—197.

— — ; SATO, S.; NAGAO, M.; YAHAGI, T.; MATSUSHIMA, T.; SEINO, Y.; TAKEUCHI, M.; KAWACHI, T.; 1976: Overlapping of carcinogens and mutagens. In: MAGEE, P. N.; TAKAYAMA, S.; SUGIMURA, T.; MATSUSHIMA, T. (Eds.): Fundamentals in Cancer Prevention. University Park Press, Baltimore, MD, pp. 191—215.

TAMURA, G.; GOLD, C.; FERRO-LUZZI, A.; AMES, B. N.; 1980: Fecalase: A model for activation of dietary glycosides to mutagens by intestinal flora. Proc. Natl. Acad. Sci. U. S. A. **77**, 4961—4965.

TOTH, B.; BOREISHA, I.; 1969: Tumorigenesis with isonicotinic acid hydrazide and urethane in the Syrian golden hamsters. Eur. J. Cancer **5**, 165—171.

TUYNS, A. J.; 1970: Cancer of the oesophagus: further evidence of the relation to drinking habits in France. Int. J. Cancer **5**, 152—156.

— — ; 1979: Epidemiology of alcohol and cancer. Cancer Res. **39**, 2840—2843.

UMEZAWA, K.; MATSUSHIMA, T.; SUGIMURA, T.; HIRAKAWA, T.; TANAKA, M.; KATOH, Y.; TAKAYAMA, S.; 1977: *In vitro* transformation of hamster embryo cells by quercitin. Toxicol. Lett. **1**, 175—178.

U. S. A. Federal Regist. 1972. **37** (149) 15426 (August 2, 1972).

WALKER, E.; CASTEGNARO, J. J.; LOQUET, E.; LETALAER, J. Y.; 1979: Investigation on etiologic factors in esophageal cancer in western France: Chemical studies and mutagenic effect on alcoholic beverages. In: THATCHER, N. (Ed.): Proc. 12th Int. Cancer Congr. Pergamon Press, Oxford, pp. 327—333.

WARWICK, G. P.; HARINGTON, J. S.; 1973: Some aspects of the epidemiology and aetiology of oesophageal cancer with particular emphasis on the Transkei, South Africa. Adv. Cancer Res. **17**, 81—85.

WATSON, W. A. F.; 1982: The mutagenic activity of quercitin and kaempferol in *Drosophila melanogaster*. Mutat. Res. **103**, 145—147.

WOLLENWEBER, E.; DIETZ, V. H.; 1981: Occurrence and distribution of free flavonoid aglycones in plants. Phytochemistry **20**, 869—932.

World Health Organization; 1972: Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. In: WHO Food Additives Series No. 5, pp. 52—59. World Health Organization, Geneva, Switzerland.

WÜRDIG, G.; SCHLOTTER, H. G.; 1967: SO_2-Bildung in gärenden Traubenmosten. Z. Lebensm.-Untersuch. Forsch. **134**, 7—13.

Eingegangen am 18. 4. 1986

Prof. Dr. R. E. SUBDEN
Department of Microbiology
University of Guelph
Guelph, Ontario
Canada N1C 2W1