studies (International Food Irradiation Project, 1979, 1981) that demonstrated the safety of irradiated subtropical fruits such as the mango.

**Registry No.** I, 26345-59-5; II, 1684-29-3; III, 57538-80-4; IV, 2092-61-7; V, 26188-06-7; VI, 28057-58-1; glucose, 50-99-7; fructose, 57-48-7; sucrose, 57-50-1; maltose, 69-79-4; ribose, 50-69-1; glyoxal, 107-22-2; crotonaldehyde, 4170-30-3; 2-deoxyribose, 1724-14-7; 2-deoxyglucose, 154-17-6; methyl vinyl ketone, 78-94-4.

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# Mutagenicity of Extracts of Some Vegetables Commonly Consumed in the Netherlands

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An assessment was made of the mutagenic properties of six vegetables commonly consumed in the Netherlands. Extracts were screened in the *Salmonella typhimurium* strains TA98, TA100, and TA1537 by using the standard protocol. In addition, extracts of the gut flora (GFE) of rats were used as a metabolizing system. In total 27 cultivars grown under known and identical conditions were tested. Cultivars of lettuce, paprika, and rhubarb were mutagenic in TA98 in the presence of GFE. String beans were mutagenic in TA98 and TA100 with GFE. Rhubarb was also mutagenic in TA1537 when tested with liver homogenate. Spinach and Brussels sprouts were found negative. The mutagenic vegetables showed marked intercultivar variations with respect to their mutagenic properties. Evidence is obtained that quercetin glycosides are mainly responsible for the mutagenicity of lettuce and string beans. The mutagenicity of rhubarb in TA1537 is caused by emodin.

Identification of mutagenic factors in the environment is of concern because they may represent an important health hazard to man and other organisms. It is well documented that certain mutagenic chemicals play a predominant role in the etiology of cancer (e.g., McCann et al., 1975; Sugimura et al., 1981) and perhaps of artherosclerosis (Benditt, 1977; Bond et al., 1981). Moreover, it is expected that exposure of the population to mutagenic chemicals may result in congenital disorders including an increased predisposition for cancer as well as genetic diseases brought about by enzyme deficiencies or other anomalies. A consideration of mutagens in food is of special concern as, according to the results of various epidemiological studies, nutritional factors seem to be of considerable importance with regard to the etiology of cancer (Doll and Peto, 1981). This hypothesis is further supported by experimental studies which show that dietary variations and the presence of nutritional and other chemical factors in food markedly influence the tumor incidence in experimental animals (Visek et al., 1978). The chemical factors identified so far include carcinogens and/or mutagens, like mycotoxins, *N*-nitroso compounds, and pyrolysis products, flavonoids, and various modulating factors such as promoters and anticarcinogens.

The present study deals with the occurrence of mutagens in commonly consumed vegetables. Various studies already show that food plants contain mutagens. Ever since the work of Auerbach and Robson (1944) mutagenicity has been reported for a large variety of other food products of plant origin (Miller and Miller, 1976; Sugimura and Nagao, 1979; Nagao et al., 1979; Takahashi et al., 1979; Cheng et al., 1980; Stich et al., 1981; Lu et al., 1981; Uyeta et al., 1981; Ivie et al., 1981). However, until now, no systematical screening of food plants has been reported. As it was suspected that the mutagenic properties of crops might show considerable regional variation as a consequence of the variability of growing conditions and cultivars, special attention was given to intercultivar variation in mutagenicity of the vegetables investigated.

## MATERIALS AND METHODS

**Chemicals.** Quercetin dihydrate was obtained from Fluka AG (West Germany), emodin from Sarsyntex (France), and rutin from Riedel—de Haën AG (West Germany). Sodium azide, L-histidine, and dimethylnitrosamine were purchased from Merck Schuchardt (West Germany). Ethidium bromide was from BDH Chemicals, Ltd. (England). Dimethyl sulfoxide ($Me_2SO$) and all other solvents were analytical grade. The NADPH-generating

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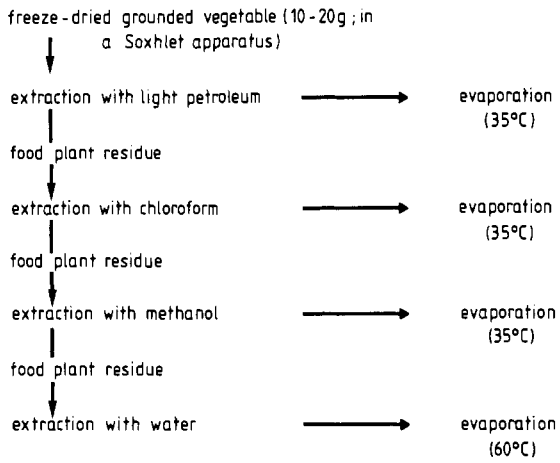


**Figure 1.** Extraction scheme (the respective extracts were evaporated to dryness in a rotary evaporator; the residues obtained in this way were dissolved in $Me_2SO$ and tested for mutagenicity).

system contained the cofactors NADP and glucose 6-phosphate from Boehringer (West Germany). Aroclor 1254 was obtained from Monsanto (Unites States).

**Source and Treatment of the Vegetables.** The origin and treatment of cultivars of six vegetables are summarized in Table I. All vegetables were washed and treated as is most commonly practiced in The Netherlands. Nonedible parts were removed. For each vegetable intercultivar variation was studied with crops that were grown, harvested, and treated under identical conditions.

The vegetables were stored at −45 °C before freeze-drying. The freeze-dried material was stored at room temperature under nitrogen in the presence of silica gel in plastic airtight boxes in the dark. The extraction and mutagenicity assessment were carried out within 3 months after freeze-drying. The freeze-dried products were treated as follows. The samples were ground and homogenized with an Ato-mix blender (Measuring & Scientific Equipments, Ltd., England). Thereafter, the material was extracted by Soxhlet extraction according to the schedule illustrated in Figure 1 and Table II. Per extraction, 400 mL of solvent was used. After extraction the solvent was decanted and a new portion of 400 mL of the next solvent was put into the Soxhlet apparatus. The extracts were evaporated to dryness in a rotary evaporator under standardized temperature conditions (Figure 1). The residues obtained were dissolved in $Me_2SO$ and subjected to the mutagenicity assays. The total amount of vegetables that could be tested per plate was limited by the ultimate solubility of the residue and/or by the toxicity of the extracts. Per plate, 0.1 mL of $Me_2SO$, containing the residue, was applied.

The free histidine content of methanol and water extracts was assessed with an automatic amino acid analyzer (Beckmann Multichrome-CR 55). If an extract was found mutagenic and did contain histidine, a purification procedure was carried out to exclude the histidine. This was achieved in all cases by dissolving the respective residue into water. This water phase was subsequently extracted 3 times with equal volumes of ethyl acetate at pH 2 (HCl, 1 N). The ethyl acetate was then evaporated, and the residues obtained, the organic acidic fractions, were dissolved in $Me_2SO$ and tested for mutagenicity. The fractions obtained in this way always contained less than 15 μg/mL histidine, resulting in an addition of less than 1.5 μg of histidine/plate.

**Mutagenicity Assays.** The mutagenicity of the various extracts was tested in the *Salmonella*/microsome assay (Ames et al., 1975) with several modifications. Histidine and biotine were not added to the top agar but to the bottom agar. Secondly and most essential next to liver homogenate, gut flora extract (GFE) from rats was applied as a metabolizing system. GFE was used to detect mutagenic aglycons of nonmutagenic glycosides. In this study tester strains TA98 and TA100 and in some specific cases TA 1537 were used. The bacteria were grown in Oxoid No. 2 nutrient broth, enriched with 10 μg/mL L-histidine, until the cultures had reached a density of $2 \times 10^9$ bacteria per mL (as determined as optical density at 700 nm). Liver homogenate from Aroclor 1254 pretreated male Wistar rats was prepared as described by Ames et al. (1975). The 9000*g* supernatant was stored under liquid nitrogen. The S9 mix (S9) used contained 30% liver homogenate; further composition was as described by Ames et al. (1975).

GFE was prepared in essentially the same way as described by Brown and Dietrich (1979). Female Wistar rats (3–9 months old) were decapitated and the cecum was removed. The cecal content obtained was mixed with ice-cold Krebs–Ringer 0.25 M phosphate buffer (pH 7.4). Per gram of cecal content, 10 mL of this buffer was added. The suspension was sonicated for 6 min (Sonifier B12 cell disrupter) at 80 W (10-s sonication followed by 10-s cooling on ice) and centrifugated at 13000*g* for 15 min at 4 °C. The supernatant was filtered (Millipore, 0.45 μm) and stored at −196 °C. GFE was filtered once again over a 0.2-μm filter (Millipore) just before application. The GFE (0.2 mL) was mixed with the bacteria (0.1 mL), Vogel Bonner medium A (0.5 mL), the test compound (0.1 mL), and the top agar (2 mL). The histidine content of the GFE was less than 12.5 μg/mL. The activity of every batch of GFE was checked by determining the mutagenicity of rutin, a glycoside of quercetin which is only mutagenic after hydrolysis of the glycoside.

The extraction procedure was carried out once for each vegetable, and the extracts obtained were tested in two independent experiments. Each experiment was carried out in triplicate. The averages of these experiments were calculated. An extract was designated mutagenic if the induced number of revertants was at least twice as high as the spontaneous (solvent control) number of revertants. Spontaneous numbers of revertants are substracted for reasons of comparison. The amounts of freeze-dried vegetables represented as extract per plate are given in the respective tables. Test results were found acceptable if the solvent control number of revertants was within the ranges of 20–70 for TA98, 80–220 for TA100, and 5–25 for TA1537.

## RESULTS AND DISCUSSION

**Mutagenicity of Extracts of Lettuce.** At first observations were made on one cultivar of lettuce (Valentine). Petroleum ether, methanol, and water extracts (see Table I) were tested in tester strains TA98 and TA100 without an exogenous activation system, in the presence of S9, and in the presence of GFE. The methanol and water extracts showed mutagenic activity for TA98 in the presence of GFE. This mutagenic activity could be concentrated in the methanol extract if extraction was carried out under reflux conditions (3 times 3 h, 20–30 g of freeze-dried lettuce to 400 mL of methanol). To exclude the effects of histidine present in the methanol extract, a simple purification procedure was carried out as described under Materials and Methods. More than 75% of the mutagenic activity was recovered in the acidic fraction obtained. To test the reproducibility of the results, three different cultivars of lettuce (designated A, B, and C) were extracted 3 or 4 times and the methanol extracts obtained were tested for mutagenicity in TA98 in the presence of GFE.

Table I. Types of Cultivars, Origin, Treatment, and Harvest Dates of the Vegetables Used for "Screening" on Mutagens

| vegetable common name | botanical name | types of cultivars | plant family | growth condition | harvest date | treatment before freeze-drying |
|---|---|---|---|---|---|---|
| paprika | *Capsicum annum* L. convar. *grossum* (L.) Terpó | Bruinsma Wonder red<br>Bruinsma Wonder green<br>Goldstar-yellow | Solanaceae | greenhouse | 6/22/81 | washed and sliced |
| lettuce | *Lactuca sativa* L. var. *capitata* | Valentine | Compositae | greenhouse | 5/16/79 | washed |
| | | Renate<br>Dandy<br>Sonate<br>Ravel<br>Deci Minor | | | 1/10/80 | |
| string beans | *Phaseolus vulgaris* L. | Romore | Papilionaceae | open air | 7/24/79 | washed, sliced, and cooked for 25 min |
| | | Romore<br>Infra<br>Helda<br>Hazet | | | 9/2/80 | |
| spinach | *Spinacia oleracea* L. | Nores | Chenopodiaceae | open air | 7/27/79 | washed and cooked for 5 min |
| | | Wolter<br>Polka<br>Spinoza<br>Sg 504<br>Sg 503 | | | 9/2/80 | |
| rhubarb | *Rheum rhabarbarum* L./ *rhaponticum* L. | Goliath<br>Paragon<br>Timperley Earley<br>Champagne Rood | Polygonaceae | open air | 7/25/79 | washed, sliced, and cooked for 5 min |
| Brussels sprouts | *Brassica oleracae* L. var. *gemarifera* (DC) Schulz | Cor Valiant | Cruciferae | open air | 9/20/78 | washed and cooked for 15 min |
| | | Rampart<br>Predora<br>Lunet<br>Cor Valiant | | | 12/9/81 | |

Table II. Extraction Specifications

| vegetable | extraction time, h, using solvent: petroleum ether | chloroform | methanol | water |
|---|---|---|---|---|
| rhubarb | 2 | | 3 times 3 | 3 |
| lettuce[a] | 2 | | 3 times 3 | 3 |
| paprika | 3 | 4.5 | 3 | 3 |
| string beans | 2 | 4 | 2 times 3 | 3 |
| spinach | 3 | 3 | 3 times 3 | 3 |
| Brussels sprouts | 3 | 3 | 3 | 3 |

[a] Only the lettuce cultivar Valentine was extracted in this way; the extraction procedure used for comparison of the mutagenicity of the different cultivars of lettuce is described under Results and Discussion.

Table III. Mutagenicity of Methanol Extracts of Five Cultivars of Lettuce toward TA98

| cultivar[a] | amount of lettuce extract, mg/plate | revertants/plate: -GFE | +GFE |
|---|---|---|---|
| Sonate | 197 | 12 | 173[c] (132[b]) |
| Ravel | 146 | 6 | 41[c] (42) |
| Renate | 121 | 10 | 279[c] (346) |
| Dandy | 164 | 6 | 185[c] (169) |
| Deci Minor | 167 | 11 | 109[c] (98) |

[a] All extracts contained less than 0.5 μg/plate histidine. [b] The numbers in parentheses correspond with the calculated numbers expressed as revertants per 150 mg of lettuce per plate. [c] Mutagenic response.

The following averages were observed (spontaneous numbers are subtracted): A, 160 ± 35 (SD; $n$ = 3); B, 216 ± 42 ($n$ = 3); C, 79 ± 15 ($n$ = 4). These results indicate that the observed mutagenic effects are reproducible and that the variation for the different extractions amounts to 20%.

To investigate intercultivar differences, five cultivars were extracted with light petroleum and subsequently with methanol as described above. The methanol extracts obtained were tested with TA98 with and without GFE. The results (Table III) show that the mutagenicity among the cultivars varies considerably, up to factor 7 if one compares the cultivars Renate and Ravel. If the number of revertants are standardized to 150 mg of freeze-dried lettuce/plate, this variation is even more pronounced.

**Mutagenicity of Extracts of String Beans.** The string beans cultivar Romore, harvested in 1979 (see Table I) showed mutagenic activity extractable in methanol (Table IV). Mutagenic activity could only be recorded in the presence of GFE. More than 75% of the mutagenic activity could be recovered from the organic extract of the acidic fraction prepared from the methanol extract.

To investigate intercultivar variation, four different cultivars were collected during the summer of 1980. At that time all the string beans available showed small necrotic patches, most likely related to the unfavorable weather conditions. Despite this they were still considered fit for human consumption. The same symptoms were observed on string beans available on the market. Mutagenicity was only observed in the methanol extracts if tested in the presence of GFE (Table V). Mutagenic activity was observed both in TA98 and in TA100. It should be noted that the extract containing the least amount of histidine was found to be the most mutagenic. Again a striking difference is found between the mutagenic activity of the different cultivars. Intercultivar variations

Table IV. Mutagenic Activity of Extracts of the String Bean Cultivar Romore (Harvest Date 1979)

| extract[a] | revertants/plate: TA98 | | | TA100 | | |
|---|---|---|---|---|---|---|
| | | +S9 | +GFE | | +S9 | +GFE |
| petroleum ether | -13 | 16 | 1 | -22 | -20 | -19 |
| chloroform | 1 | -9 | 12 | 28 | -18 | -19 |
| methanol[b] | 24 | 4 | 262[d] | 32 | 6 | 40 |
| water[c] | 25 | 6 | 29 | 25 | | 17 |

[a] Extracts of 400 mg of freeze-dried string beans added per plate; extraction as described in Table II. [b] 8.2 μg of histidine/plate. [c] 9.0 μg of histidine/plate. [d] Mutagenic response.

Table V. Mutagenicity of Methanol Extracts of Four Cultivars of String Beans in the Presence of GFE

| cultivar[a] | histidine content of the extracts, μg/plate | revertants/plate: TA98 | TA100 |
|---|---|---|---|
| Romore | 4.4 | 247[b] | 280[b] |
| Infra | 10.7 | 257[b] | 163[b] |
| Hazet | 8.5 | 408[b] | 270[b] |
| Helda | 1.5 | 658[b] | 442[b] |

[a] Extracts of 400 mg of freeze-dried string beans added per plate; extraction as described in Table II. [b] Mutagenic response.

amounted to maximal 2.6 for TA98 and 2.7 for TA100.

**Mutagenicity of Extracts of Paprika.** Three different types of paprika, two of them are cultivar varieties, were tested for mutagenicity. The red and green paprikas investigated are the ripened and unripe form of the cultivar Bruinsma Wonder.

Mutagenic activity was only found in the methanol extracts toward TA98 in the presence of GFE (Table VI). Almost 100% of the mutagenic activity of the paprikas could be concentrated in the acidic organic fraction prepared from the methanol extracts. It is noteworthy that the methanol extract of red paprikas was found to exhibit a smaller mutagenic effect than the extract of the green paprika.

**Mutagenicity of Extracts of Rhubarb.** Four cultivars of rhubarb were investigated. In two cases mutagenic activity was observed toward TA98 in the methanol extracts in the presence of GFE (Table VII). About 75% of the mutagenic activity observed in the methanol extracts could be concentrated in the acidic organic fraction. Maximum intercultivar variation is more than 10-fold.

From the literature it is known that rhubarb may contain anthraquinones both as aglycons and glycosides. Some of these anthraquinones are mutagenic toward TA1537. Therefore, extracts of rhubarb were also tested with this strain. At first petroleum ether, chloroform, methanol, and water extracts of the cultivar Paragon were tested (extraction times 2, 2, 3, and 3 h, respectively). Weak mutagenic activity was observed in the first three extracts in the presence of S9. This mutagenic activity could be concentrated in the combined petroleum ether and chloroform extracts if extraction times were prolonged to 3 h. For all cultivars a mutagenic response was observed in the presence of S9 (Table VII). Again a marked intercultivar variation was observed. If the standardized figures are used, the maximum intercultivar variation amounts to 8.8.

**Absence of Mutagenicity in Brussels Sprouts and Spinach.** The Brussels sprouts cultivar Cor Valiant was

Table VI. Mutagenicity of Methanol Extracts of Different Cultivars of Paprika

| cultivar[a] | histidine content of the extracts, μg/plate | revertants/plate TA98 | TA98 +GFE | TA98 +S9 | TA100 | TA100 +GFE | TA100 +S9 |
|---|---|---|---|---|---|---|---|
| Goldstar | 2.2 | 17 | 90[b] | 11 | 6 | 19 | 15 |
| Bruinsma Wonder green | <0.5 | 8 | 198[b] | −3 | −2 | 52 | 27 |
| Bruinsma Wonder red | <0.5 | 12 | 76[b] | 8 | 11 | −4 | 68 |

[a] Extracts of 200 mg (Goldstar and Bruinsma Wonder red) or 160 mg (Bruinsma Wonder green) per plate; extraction as described in Table II. [b] Mutagenic response.

Table VII. Mutagenicity of Methanol Extracts and Combined Petroleum Ether and Chloroform Extracts of Four Cultivars of Rhubarb

| cultivar[a] | revertants/plate methanol extract TA98 | TA98 +GFE | TA98 +S9 | TA100 | TA100 +GFE | TA100 +S9 | combined petroleum ether and chloroform extract TA1537, +S9 |
|---|---|---|---|---|---|---|---|
| Paragon | 14 | 126[d] | 2 | 15 | 42 | −13 | 120[b,d] |
| Goliath | 13 | 13 | 14 | 16 | −39 | −11 | 20[b,d] |
| Champagne Rood | 5 | 27 | 9 | −2 | 30 | 5 | 23[b,d] |
| Timperley Earley | 26 | 157[d] | 3 | 22 | 35 | −9 | 116[d] (176[c]) |

[a] Extract of 132 mg of freeze-dried rhubarb added/plate; extraction as described in Table II; only the methanol extract of Paragon was analyzed on histidine (9.0 μg/plate). [b] Extract of 200 mg of freeze-dried rhubarb added/plate. [c] The number in parentheses corresponds with the calculated number as expressed as revertants per 200 mg of freeze-dried rhubarb. [d] Mutagenic response.

investigated extensively for the occurrence of natural mutagens. All extracts tested were negative in strains TA98 and TA100. The methanol and water extracts contained high amounts of histidine (10–25 μg/plate). Even at these high histidine levels no significant increases in the number of revertants were observed. The alkaline and acidic fractions prepared from the water and methanol extracts were also negative. The acidic fractions of methanol and water extracts of three other cultivars, Rampart, Lunet, and Predora, were also tested with TA100 and TA98 and all were found to be negative (extracts of 160–500 mg of freeze-dried product/plate were tested). Six cultivars of spinach (see Table I) did not show mutagenic effects under the test conditions applied (extracts of 500 mg of freeze-dried product/plate were tested).

**Identification of Mutagenic Compounds.** The mutagenic methanol extracts of the lettuce cultivar Renate and the string beans cultivar Romore were purified. This was achieved by liquid chromatography using polyamid and reversed-phase $C_8$ material as adsorbents (details of the purification procedure will be published elsewhere). Chemical hydrolysates of the obtained purified mutagenic fractions contained quercetin, according to HPLC analysis, in amounts that can account for the mutagenic effects observed.

The combined petroleum ether and chloroform extract of the rhubarb cultivar Paragon was purified by liquid chromatography with silica gel as the adsorbent. The finally obtained mutagenic fraction contained a single substance, according to HPLC analysis, with the same retention time as the anthraquinone emodin. This finding was confirmed with mass spectrometry. The cultivar Paragon did contain about 35 μg of emodin in a free form per g of freeze-dried material.

**Role of Histidine in the Outcome of the Mutagenicity Assays of Vegetable Extracts.** The presence of histidine in samples that are tested in the *Salmonella*/microsome assay may influence the outcome of the mutagenicity trials. The presence of histidine will mimic the induction of revertants to a certain degree. Since a number of extracts of food products do contain considerable amounts of this amino acid, we have investigated the effect

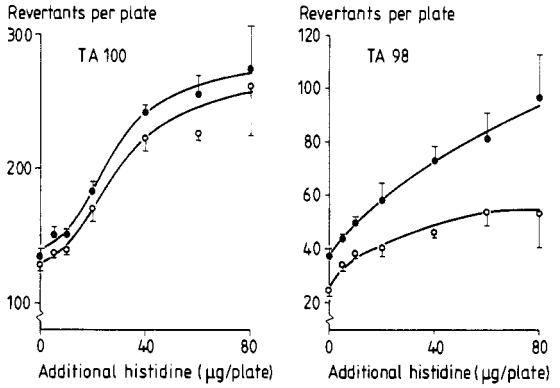


**Figure 2.** Influence of additional histidine on the spontaneous number of revertants: all values are averages of four experiments; (○) without S9; (●) with S9; (vertical bar) SEM.

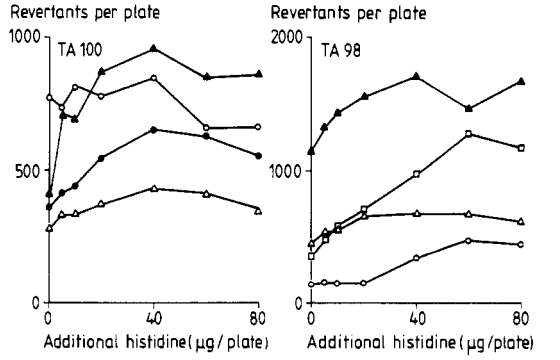


**Figure 3.** Influence of additional histidine on the mutagenic effect of various mutagens: (○) $NaN_3$, 1 μg/plate; (●) DMN, 10.1 mg/plate, +S9; (□) EB, 50 μg/plate, +S9; (△) quercetin, 25 μg/plate; (▲) quercetin, 25 μg/plate, +S9.

of additional amounts of histidine on spontaneous as well as on the induced number of revertants (Figure 2 and 3). In general, additional amounts of histidine higher than 20 μg/plate resulted in plates with very dense lawns of background growth and irregular patterns of revertant colonies. Histidine levels of 20 μg/plate or less, on top of

the normal histidine content (15 μg/plate), never resulted in a doubling of the normal spontaneous number of revertants.

The effect of histidine on various mutagens is not additional (Figure 3). For every compound tested a different effect is observed. For all compounds the prsence of additional histidine exaggerated the mutagenic effect. In the case of ethidium bromide (EB) a magnification of the mutagenic effect is observed in TA98, in the presence of liver homogenate. Only a small effect is found with sodium azide ($NaN_3$) in TA100. In the case of quercetin small effects are observed in the absence of S9 whereas more pronounced increases in mutagenic effects especially in TA100 are observed in the presence of S9. With dimethylnitrosamine (DMN) an increase in the number of revertants is observed, up to a level of additional histidine of 40 μg/plate. Above this level the curve is deflecting, as with almost all the other compounds tested. These observations are similar to those described by Ames et al. (1975) and Friederich et al. (1982).

Mutagenic effects of mutagens present in histidine containing samples may be overestimated. If possible, purification procedures should be carried out to avoid histidine. Samples resulting in addition of more than 20 μg of histidine/plate cannot be considered because of irregular growth patterns of the colonies on the test plates. It should also be kept in mind that exogenous activation systems like S9 and GFE do contain small amounts of histidine. Application of S9 and GFE in this study never resulted in addition of more than 3 and 2.5 μg of histidine/plate, respectively.

## GENERAL DISCUSSION

In this study six vegetables were investigated on the occurrence of natural mutagens. Four vegetables, lettuce, string beans, paprika, and rhubarb, were found to be mutagenic. It should be noticed that the lack of mutagenicity of the other two vegetables, spinach and Brussels sprouts, is no proof of the absence of mutagens. Mutagens may be present at low concentrations or may simply not react in the test systems used. Mutagenic activity may also be masked by the presence of compounds with antimutagenic activity.

At this moment it is not possible to assess the toxicological importance of the occurrence of the natural mutagens in vegetables as reported in this study. Quercetin, the compound that is at least partly responsible for the mutagenic activity observed in lettuce and string beans, has been found positive in a number of in vitro mutagenicity assays (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; Sugimura, 1979; Maruta et al., 1979; Meltz and MacGregor, 1981) and in vitro cell transformation assays (Umezawa et al., 1977; Meltz and MacGregor, 1981). The data on the carcinogenicity of quercetin are conflicting. Pamukcu et al. (1980) reported that quercetin is a potent inducer of tumors in rats. However, Ambrose et al. (1952), Saito et al. (1980), Hirono et al. (1981), Hosaka and Hirono (1981), and Morino et al. (1982) found that quercetin was noncarcinogenic in mice, rats, and golden hamsters at even higher dose levels than those tested by Pamukcu. The data on in vivo mutagenicity of quercetin, which may be important in relation to genetic diseases, aging, and artherosclerosis, are to scarce to draw conclusions but cannot be excluded.

In this study emodin was found to be responsible for the mutagenicity of light petroleum and chloroform extracts of rhubarb. Emodin has been reported to be positive in the *Salmonella*/microsome assay by various authors (Brown and Brown, 1976; Wehner et al., 1979; Tikkanen et al., 1982; Levin et al., 1982). Although there is structural similarity with carcinogens like luteoskyrin and daunamycin, emodin has not yet been investigated on carcinogenicity.

The four vegetables, that were found to contain mutagens, all showed marked intercultivar variations. Because the respective cultivars were grown under identical conditions and treated with standard procedures, the conclusion can be drawn that the observed differences are related to the intrinsic mutagenic properties of the vegetables. Differences in intrinsic mutagenic properties may originate from plant breeding to obtain more disease-resistant vegetables. By such efforts higher amounts of preinfectional defense substances may be introduced in crops. The ability to produce phytoalexins upon fungal contamination or other stress conditions may also be increased. A third consequence of plant breeding can be the introduction of "new" natural chemicals into plant species. In this context it may be relevant to draw attention to the recently developed lettuce cultivar resistant to the leaf aphid *Nasonovia ribis nigri* (Eenink et al., 1982). The introduced resistance, a result of crossing the cultivated lettuce (*Lactuca sativa*) with a wild lettuce type (*Lactuca virosa*), seems to be caused by a compound or compounds present in the phloeem of the lettuce. The developed resistant cultivar did not show more mutagenic properties than the native lettuce cultivar (van der Hoeven and Fennis, 1982). An example of probable involvement of mutagenic properties of phytoalexins may be formed by the results that were obtained in this study with string beans. The cultivars that were used in this study all showed necrotic patches as a result of specific stress conditions. In contrast with the other vegetables found mutagenic in this study, a mutagenic response was observed in TA100 (Table VI). Such a mutagenic response was not observed with the string beans cultivar Romore, harvested in 1979, which showed no signs of stress damage (Table V). As a result of the unfavorable climate conditions in 1980, phytoalexins may have been formed in the cultivar Romore and in the other cultivars investigated which contributed to the mutagenicity observed for TA100.

In the light of the fact that plant breeding in general is nowadays concentrated on the introduction of disease-resistant cultivars, these activities should receive more toxicological attention in the future.

**Registry No.** Emodin, 518-82-1; L-histidine, 71-00-1; EB, 1239-45-8; $NaN_3$, 26628-22-8; DMN, 62-75-9; quercetin, 117-39-5.

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# Determination of Nitrite and Volatile Nitrosamines in Animal Diets

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Animal diets (chow, agar, and casein) similar to those used in two nitrite feeding studies that reported an increase in the incidence of lymphomas in rats were analyzed over a 14-day period. Sodium nitrite was added to the chow diet at levels of 0, 1000, and 2000 ppm, to the agar diet at 0, 500, 1000, and 2000 ppm, and to the casein diet at 0 and 1000 ppm. Trace amounts (0.5 to <1.0 ppb) of volatile nitrosamines were found in the agar and casein diets. The levels in the chow diet were significantly higher (5–45 ppb) and increased with the duration of storage as well as with the concentration of added nitrite. The predominant nitrosamine found in the chow diet was *N*-dimethylnitrosamine (NDMA); chow to which no nitrite had been added contained an average of 4 ppb of NDMA. Measurements of the nitrite levels in these diets indicate that the amount of nitrite found was substantially less than the amounts that had been added, possibly because of loss, degradation, or interaction with dietary components.

Two recent studies reported that the addition of sodium nitrite to the diet or drinking water of rats increased the incidence of lymphoreticular tumors (Shank and Newberne, 1976; Newberne, 1979). The object of the experimental approach was to determine whether nitrites per se and not nitrosamines induced cancer in rats. Since direct documentation concerning the presence or absence of nitrosamines in the original experimental diets was not obtainable, similar diets were reconstructed and treated with nitrite to determine whether or not volatile nitrosamines were formed (during mixing or storage) in the diets fed to the animals. We have therefore measured the volatile nitrosamines and nitrite in these diets, stored in the same way as the diets used in the original feeding studies.

## EXPERIMENTAL SECTION

Most nitrosamines are potent carcinogens in experimental animals (Magee et al., 1976); extreme care should be exercised in their storage, handling, use, and disposal.

**Chemicals.** All solvents were distilled in glass grade (Burdick and Jackson Laboratories, Muskegon, MI). Nitrosamine standards were diluted from stock solutions supplied by the Thermo Electron Analytical Services Laboratory (Waltham, MA). These solutions were calibrated by comparison with standards supplied by the Food and Drug Administration (FDA). Other chemicals (reagent grade) were supplied by Chemical Dynamics Corp., South Plainfield, NJ (±-$\alpha$-tocopherol), Fisher Inc., Fairlawn, NJ (ammonium sulfamate), Aldrich Chemical Co., Milwaukee, WI (sulfanilamide), and J. T. Baker Chemical Co., Phillipsburg, NJ [*N*-(1-naphthyl)ethylenediamine].

**Diets.** For this 14-day study, diets were prepared at the Massachusetts Institute of Technology, Cambridge, MA. Two kilograms of each of three types of diet was made as described by Wogan and Newberne (1967) and Newberne (1979). These included dry reground pellets of a commercial rodent chow (Agway-Charles River RMH 3000), a dry casein diet, and a wet agar diet prepared in the same way as the casein diet except that agar (1.17%) and water (50%) were added, producing a gel instead of a powder.

Sodium nitrite was added to the diets at the following levels: chow diet, 0, 1000, and 2000 ppm; agar diet, 0, 500, 1000, and 2000 ppm; casein diet, 0 and 1000 ppm. In the

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