Irradiated Strawberries—Chemical, Cytogenetic, and Antibacterial Properties

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γ-Irradiated fresh whole strawberries were investigated. Doses of 0.15 and 0.3 Mrad produced no changes in the sugar content of dialysates and freeze-dried powders. Gas chromatography and mass spectrometry of volatile fractions revealed minimal effects of 0.2 Mrad. Volatiles from 1.5 Mrad samples revealed eight compounds not detected in our nonirradiated samples. One new compound was tentatively identified as methoxyacetaldehyde. Carbonyl compounds are found in dialysates from irradiated (0.2–3.0 Mrad) strawberries with initial G(carbonyl) = 2.2. When the corresponding dialysates are autoclaved in open vessels, the carbonyl levels dropped about 50% at doses >0.5 Mrad. Dialysate preparations (0–1.5 Mrad) did not inhibit growth of *Salmonella typhimurium* LT2. Irradiation made no difference (p = 0.01) in the number of chromosomal or chromatid aberrations observed *in vitro* or *in vivo*. An increase in chromatid aberrations *in vitro* was produced by unsealed autoclaved dialysates (0.5 and 1.5 Mrad). Overall, we find that unlike irradiated dilute solutions of sugars, no significant amounts of cytotoxic agents including α,β-unsaturated carbonyls are formed in low dose (≤0.2 Mrad) strawberries.

The radiation pasteurization (radurization) treatment of foods as a means of prolonging storage life is becoming an accepted procedure, especially abroad (Kraybill and Whitehair, 1967; Shea, 1971). There has been special interest in the radurization of strawberries at radiation doses of 200 krad to inhibit grey mold formation (Maxie and Sommer, 1968; Shibabe *et al.*, 1967). However, before an irradiated food can be cleared for human consumption, it must pass the usual toxicity tests and a battery of CMT tests (C = clastogenic [chromosome-breakage], M = mutagenic, and T = teratogenic) and, if possible, identify and quantitate the levels of radiolytically produced substances.

Radiolysis of irradiated carbohydrates in aqueous solution ($\sim 10^{-2}$ M) produces cytotoxic and possibly mutagenic substances based on *in vitro* tests (Holsten *et al.*, 1965; Kesavan and Swaninathan, 1971; Schubert, 1969; Shaw and Hayes, 1966). The concentration of mono- and polysaccharides, however, in strawberries is so much higher (*ca.* 8% fresh weight) than in the aforementioned aqueous solutions that the formation of cytotoxic and mutagenic agents by the indirect action of OH radicals (Schubert and Sanders, 1971) appears unlikely.

Treatment of *Vicia faba* roots with juice or puree from irradiated (200–400 krad) strawberries (Ross *et al.*, 1970) produced no significant cytological effects, as measured by the percentages of abnormal anaphases. More recently, Malling *et al.* (1971) tested irradiated strawberries which were either fresh frozen or freeze-dried for mutagenicity by the host-mediated assay procedure (Legator and Malling, 1971) and found no increase in the frequency of ad-3 mutations among heterokaryotic conidia incubated in the peritoneal cavity of rats during feeding with food containing irradiated strawberries.

Irradiation produces some chemical changes in strawberries, as indicated in a few scattered reports. These include a modest decrease in ascorbic acid with slight changes in niacin and thiamine (Maxie and Sommer, 1968), although no effect on ascorbic acid is observed at doses up to 200 krad (Clarke, 1959). At doses above 200 krad some loss in the red color of irradiated strawberries occurs as a result of destruction of the anthocyanin pigments (Horubala, 1968; Maxie and Sommer, 1968). No organic free radicals in irradiated (200 krad at 0°) strawberries were found by epr measurements (Shah *et al.*, 1966).

Our investigations include homogenates, centrifugants, distillates, and powders prepared from untreated and irradiated whole fresh strawberries and freeze-dried strawberries using chemical and biological procedures. The chemical evaluations included gas chromatographic and mass spectrometric assays. In particular, we made chemical assays for the first time of *total* carbonyl compounds produced in irradiated strawberries. However, the formation of *volatile* carbonyl compounds in irradiated foods is well known; *e.g.*, in meat and meat fats (Batzer *et al.*, 1957) and milk (Khatri *et al.*, 1966).

We have indicated (Schubert and Sanders, 1971) that the cytotoxic and clastogenic compounds formed upon γ-irradiation of dilute aqueous solutions of carbohydrates are principally dicarbonyl sugars which give rise to α,β-unsaturated carbonyl sugars upon thermal dehydration (autoclaving) or enolization. These hydroxylated compounds are characterized chemically in many ways, including: pH-dependent ultraviolet spectra; reaction with 2,4-dinitrophenylhydrazine (2,4-DNP) to yield characteristic visible absorption spectra; and nonvolatility. Heretofore, except for fats, only the volatile carbonyls produced in foods have been measured because of interferences from carbohydrates. We are able to measure total carbonyls in the presence of carbohydrates without prior separation by modification of the usual 2,4-DNP procedure (Sanders and Schubert, 1971).

The cytological studies reported here include *in vitro* and *in vivo* measurements in rats for chromosomal aberrations. In addition, we tested irradiated strawberry homogenates and extracts for antibacterial activity—a well known manifestation of irradiated carbohydrate solutions (Schubert, 1969).

EXPERIMENTAL SECTION

Irradiation. Samples were irradiated in a cobalt-60 Gammacell-220 (Atomic Energy of Canada, Ltd.) delivering 6 krad/min. The temperature in the irradiation chamber was maintained at 25 ± 1°. Radiation dosimetry was performed with a ferrous sulfate dosimeter (National Bureau of Standards, 1964) under appropriate conditions; *e.g.*, container size, volumes, etc.

Preparation of Strawberry Samples. One-hundred-and-fifty-gram samples of fresh, whole California strawberries were washed three times with distilled water and drained just prior to irradiation. Samples were irradiated in a 400-ml glass beaker sealed with Parafilm. After irradiation the calyx was removed, the sample was placed in a Waring blender, 50 ml of distilled H_2O was added, and the mixture was blended for 30 sec. Immediately after blending, 80 g of the puree was placed in a Visking dialysis bag and dialyzed in the cold (5°) for 19 hr against 100 ml of distilled

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H_2O. The dialysates, orange-colored clear solutions, were removed and placed in stoppered bottles at 5°. The dialysates obtained from strawberries irradiated at different doses were analyzed for carbonyls. Each milliliter corresponds to an extract from 0.33 g of fresh strawberries.

Irradiated whole strawberries lost color in proportion to the radiation dose, a phenomenon reflected by the decrease in an absorption peak at 496 nm. The colors varied from red in the controls to "lighter red" at 200 krad, to light pink at 600 krad, and the "very light pink" at 800 krad. Strawberries irradiated above 500 krad also lost liquid but the amounts were relatively small as long as the calyx was present during irradiation.

Moisture determinations on fresh strawberries were made by placing 15–20 g of sliced strawberries on tared aluminum weighing dishes and drying overnight at 105° to constant weight. Within experimental error, the moisture contents were practically identical for the unirradiated controls and the low dose irradiated samples: 0 krad–91.0%; 150 krad–90.8%; and 300 krad–90.4%.

Homogenates were prepared from whole strawberries, and supernatants were prepared by cold (5°) centrifugation (g = 1800) for 15 min. Depending on the particular batch of strawberries used, the pH's ranged from 3.7 to 3.9 and were unchanged by radiation.

No H_2O_2 could be detected in the centrifugants prepared from irradiated (150 or 300 krad) fresh whole strawberries. The assay was made on HCl-acidified centrifugant following dilution (1:10) with H_2O by both the titanium sulfate and iodometric procedures (Egerton *et al.*, 1954).

We obtained samples of freeze-dried strawberries, prepared in a manner described elsewhere (WARF, 1971), from a stock used for the long-term feeding studies. The strawberries used were of the Shasta variety (Salinas, Calif.) and harvested while in the pink or three-quarter ripe stage. The whole berries were irradiated at 6° and stored hard frozen until vacuum-dried at 130°F (100–300 μ) for 24 hr. Eventually the vacuum-dried strawberries were ground with a $^3/_{32}$ in. screen size Fitzmill. Three lots of vacuum-dried strawberries were used: =1, unirradiated control; =2, 150 krad irradiated; and =3, 300 krad irradiated.

Carbonyl Analyses. Total carbonyls were determined on the dialysates or supernatants, and distillates from homogenates of fresh whole strawberries by the modified 2,4-DNP method (Sanders and Schubert, 1971). Samples were spiked with known amounts of various carbonyl compounds, *e.g.*, glyceraldehyde, in order to check recovery efficiencies. In all cases, recoveries of 95–100% were consistently obtained.

Gas Chromatographic Assays and Mass Spectrometry of Nonvolatile and Volatile Constituents. Fresh whole strawberries, (Shasta variety) with 0, 150, and 300 krad, were analyzed for carbohydrates. A 600-g sample of each group of strawberries was blended in a Waring blender at 5° for 2 min. The homogenate was then centrifuged in a Sirvall refrigerated centrifuge at 2500 × g for 15 min. The supernatants (275–300 ml) were decanted and the pellets were resuspended and recentrifuged as before. The two supernatants, when combined, brought the total volume to 400 ml. A 0.20-ml aliquot of each sample was removed and dried for 30 min at room temperature under low pressure (1–2 mm) and then dried for an additional 30 min at 50°. The carbohydrates in the resulting red powder were converted to the per-*O*-trimethylsilyl ethers (Sweeley *et al.*, 1963) by the addition of 1 ml of Tri-Sil reagent (Pierce Chemical Company). The mixture was shaken and then allowed to stand at room temperature for 30 min, at which time all of the red residue had dissolved. The resulting solution was analyzed for carbohydrates by gas chromatography using a Perkin-Elmer Model 900. We used a 5 ft × $^1/_8$ in. OV-1 column, and the column temperature was programmed for 100–250° at 5°/min.

The carbohydrates in a 0.20-g sample of the freeze-dried strawberries were converted to per-*O*-trimethylsilyl ethers using 0.8 ml of Tri-Sil reagent. The gas chromatographic analyses were also carried out on a 3% OV-1 column, temperature programmed from 135 to 200° at 2°/min and then to 265° at 8°/min.

Gas chromatographic assays for volatiles produced by irradiation were carried out using California strawberries purchased in May 1972 in the local supermarket. The strawberries were irradiated and homogenized as described.

Ten grams of the strawberry homogenate was added to a 100-ml round-bottomed flask equipped with a short-path distillation head. The distillation apparatus was connected to three traps containing ice water, Dry Ice–methanol, and liquid nitrogen, respectively. The distillation was started at room temperature under a reduced pressure of 1 mm. During the distillation the temperature was increased to 45–50° over a period of 1 hr and the distillation was then continued at this temperature for an additional hour. The three fractions were combined for coupled gas chromatography–mass spectrometry analyses.

The aqueous distillate was saturated with sodium chloride and extracted with five 2-ml portions of cold double-distilled ether. The ether extracts were combined and dried for 30 min over sodium sulfate in the cold. The ether was decanted into a 15-ml flask and the sodium sulfate was washed with 2 ml of ether. The ether solution was distilled through an 8-in. Vigreaux column in a water bath maintained at 40–42°. Distillation was stopped just before dryness, and the flask cooled. The residue (about 0.2–0.3 ml) was transferred to a clean, teflon-stoppered vial for analysis.

Preliminary mass spectrometric analysis of the volatiles was carried out using a LKB 9000 coupled gas chromatograph–mass spectrometer equipped with a 6 ft × $^1/_8$ in. glass column packed with 15% Carbowax 20M on Gas Chrom Q. The column temperature was programmed as follows: 10 min at 65°; 65–150° at 4°/min; 30 min at 150°. The inlet and oven temperatures were set at 250°. The chromatograms were obtained using helium as the carrier gas at a flow rate of 30 ml/min. Mass spectra were obtained at 70 eV.

Biological Tests. It is possible to identify classes of compounds from the degree to which they inhibit bacterial growth; *e.g.*, *Salmonella typhimurium* LT2 (Schubert, 1970; Schubert and Sanders, 1971). The test organism is grown in a phosphate-buffered synthetic medium with glucose as the carbon source. A given volume of an irradiated or unirradiated sample is mixed with the basal medium and an inoculum of saline-washed log phase cells. Measurement of growth is made turbidimetrically. Sucrose does not contribute to the growth of the bacteria. In the experiments described here, varying volumes of extracts of irradiated strawberries were incorporated in the growth medium.

The cytogenetic effects of irradiated strawberries were measured both *in vitro* and *in vivo*. In the *in vitro* experiments, a modified short-term human lymphocyte culture was used (Moorehead and Nowell, 1964). The method employs three or four drops of blood from a finger prick incubated in 5 ml of Eagle's minimum essential spinner medium with phytohemagglutinin at 37° for 2 days. At this time, dialysates prepared from fresh whole strawberries (0 and 1.5 Mrad irradiated) were incorporated in the culture medium and the culture was incubated for an additional 24 hr, at which time the cells were treated with Colcemid and harvested. The metaphase cells are searched for structural aberrations and the chromosomal aberrations are scored in accordance with a modification (Jacobs *et al.*, 1964) of Buckton's classification. Only those mitotic cells in the search area are examined for which there is ade-

Table I. Gas Chromatographic Analysis of Sugars in Vacuum-Dried Strawberry Powders[a]

Component	Weight % (dry weight basis)		
	Unirradiated	150 krad	300 krad
Carbohydrates			
D-Fructose	21	21	20
D-Glucose	20	22	20
D-Sucrose	3.8	3.1	4.8
Total sugars	45	46	45

[a] Details of the preparation of the powders and the gas chromatographic assay are described in the Experimental Section.

Table II. Gas Chromatographic Analysis of Fresh Whole Strawberries[a]

Components	Weight % (fresh weight basis)		
	Unirradiated	150 krad	300 krad
D-Fructose	2.3	2.2	2.4
D-Glucose	2.6	2.6	2.9
D-Sucrose	0.14	0.18	0.22
Total sugars	5.0	5.0	5.5

[a] Experimental details such as column, temperature, programming, etc., are given in the Experimental Section.

quate spreading of the chromosomes. The cells are randomly scanned without regard to the presence or absence of aberrations.

The *in vivo* experiments were carried out using puree prepared from irradiated (0 and 1.5 Mrad) fresh whole strawberries. The puree was given to 11-week-old Swiss-Webster female mice by intubation three times a day for 5 days. In a similar manner, 250–300-g Sprague-Dawley male rats were given suspensions of the freeze-dried strawberry powders. In all cases, a surplus of animals was treated to allow for occasional deaths caused by perforation of the esophagus during the intubation feeding. Subsequently, cytogenetic measurements were made on cells obtained by serial aspirations of bone marrow from the femurs on three separate occasions, namely, on days 1, 5, and 7 after the completion of intubation feeding. The aspiration technique has been described in detail elsewhere (Pan and Wald, 1963; Schubert, 1969). We have applied the aspiration technique many times in the past to irradiated animals (Schubert *et al.*, 1970) and to animals receiving clastogenic chemicals, *e.g.*, benzene (Wald and Pan, 1970), and have observed positive dose-response production of chromosomal aberrations. The metaphase cells were scored in the same manner as that of the lymphocyte cultures.

RESULTS AND DISCUSSION

Gas Chromatographic Analyses of Nonvolatiles. The major carbohydrates for vacuum-dried strawberry powders were identified as D-fructose, α-D-glucose, β-D-glucose, and D-sucrose. The weight percentages of the sugars were calculated from the peak areas using calibration curves constructed from standard solutions (Pierce Chemical Company) of per-*O*-trimethylsilyl-D-fructose, D-glucose, and D-sucrose and are shown in Table I. No significant differences in carbohydrate composition were observed between the unirradiated and irradiated sample.

The chromatograms prepared from the centrifugants of unirradiated and irradiated (150 and 300 krad) fresh whole strawberries showed four major peaks. From the retention times these were identified as D-fructose, α-D-glucose, β-D-glucose, and D-sucrose in order of increasing

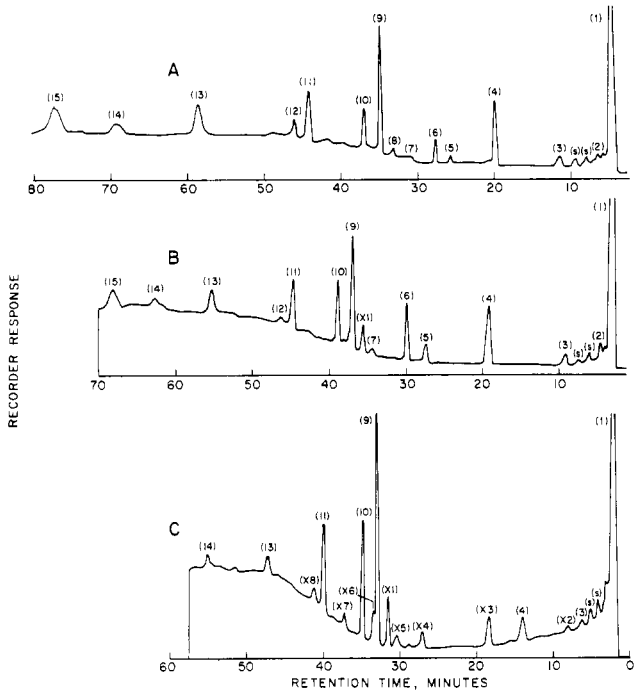


Figure 1. Gas chromatogram of volatiles obtained from: A, nonirradiated strawberries; B, irradiated strawberries (0.2 Mrad); and C, irradiated strawberries (1.5 Mrad). The chromatogram was obtained using an LKB 9000 coupled gas chromatograph–mass spectrometer on a 6 ft × 1/8 in. column packed with 15% Carbowax 20M. Temperature was programmed from 65 to 150° at 4°/min and then isothermally at 150°. Peaks assigned the same number in the three panels signify that the compounds are identical, as evidenced from the fact that their mass spectra are identical. Peaks labeled S are produced by solvent (diethyl ether) impurities. Peaks labeled X in panels B and C are new peaks not present in the gas chromatograms of the nonirradiated strawberries.

temperature. In addition, a minor peak appeared between D-fructose and α-D-glucose which is most probably α-D-mannose, but this peak was not sufficiently resolved for quantitative evaluation. The results, expressed in weight percentages, as obtained from the calibration curve, for each of the three sugars are shown in Table II. Within experimental error, no differences exist between the unirradiated and irradiated strawberries.

The levels of the three principal sugars in both the fresh whole strawberries (90% H_2O) and vacuum-dried powders were in good agreement when calculated on the same basis, *i.e.*, fresh weight or dry weight. Our values are also in good agreement with the amount of invert sugar in fresh strawberries reported by Boland *et al.* (1968) or the total "crude" carbohydrate levels reported by Watt and Merrill (1963). The "crude" carbohydrate values were obtained by calculation, taking the difference between 100 and the sum of the percentages of crude protein, crude fat, ash, and water.

Gas Chromatography and Mass Spectrometry of Volatiles. Gas chromatography was performed on the volatile compounds present in strawberries at doses of 0, 0.2, and 1.5 Mrad, respectively. The resulting gas chromatograms are shown in Figure 1, a–c.

At least 15 volatile compounds were detected in nonirradiated strawberries under the stated conditions (Figure 1a). All compounds which were tentatively identified have been previously found by other workers who analyzed volatiles from far larger samples of strawberries (McFadden *et al.*, 1965; Tressl *et al.*, 1969). These include: ethyl acetate, peak (1); 2-pentanone, peak (2); *trans*-2-hexenal, peak (6); and β-pinene, peak (8).

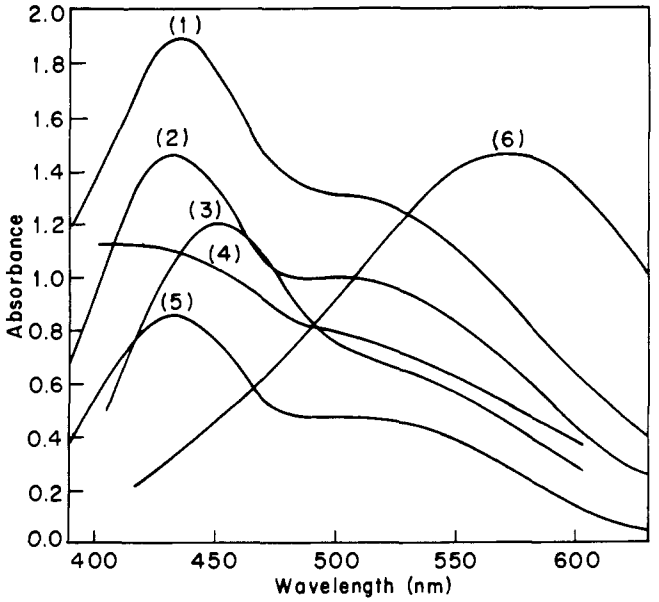


Figure 2. Visible absorption spectra of 2,4-DNP-treated dialysates prepared from fresh whole strawberries and of irradiated sucrose and model carbonyl compounds. The absorption spectra were obtained using an automatic recording SP-800 UNICAM spectrophotometer. (1) Irradiated (300 Krad) sucrose (5.8×10^{-2} *M*). (2) Difference spectrum of dialysate of irradiated (800 Krad) strawberries. (3) Autoclaved deoxyribose, 3.8 μmol/ml of unsaturated aldehyde thermal dehydration product ($CH_2OHCHOHCH{=}CHC(=O)H$). (4) Unirradiated strawberry dialysate. (5) Glyceraldehyde, 0.33 μmol/ml. (6) Glyoxal, 0.34 μmol/ml.

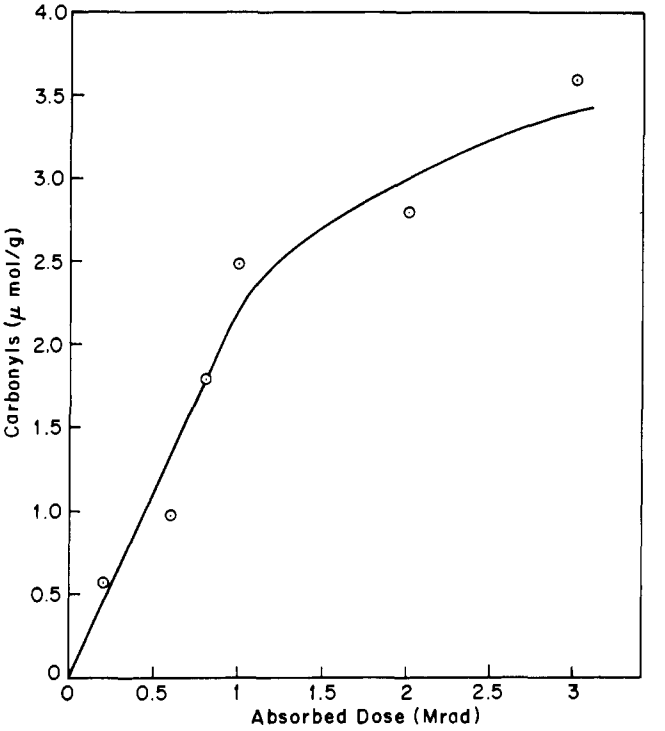


Figure 3. Carbonyls produced upon irradiation of fresh whole strawberries as a function of radiation dose. The carbonyl assays were made on the dialysates prepared from homogenized preparation of the fresh whole strawberries as described in the text. The calculation of carbonyl concentrations was based on a molar absorptivity, $\epsilon = 18 \times 10^3$, corresponding to that of glyceraldehyde.

In some cases, peaks may appear to be similar in the unirradiated or irradiated samples or to consist of a single component. However, such questions are usually resolved by mass spectrometry. Those peaks assigned the same number in Figures 1, a-c, are the same compound based on their identical mass spectra.

The gas chromatogram of the 0.2 Mrad sample (Figure 1b) is nearly the same as that of the nonirradiated sample (Figure 1a). Peak (X1), however, while having a position similar to peak (8) in the nonirradiated sample, is a different compound tentatively identified as isobutyric acid from the mass spectrum. This compound is known to be present as a minor component in the volatile fractions of strawberries (Tressl *et al.*, 1969). Our finding suggests, therefore, that irradiation at 0.2 and 1.5 Mrad produced additional amounts of isobutyric acid.

The gas chromatogram of the volatiles from 1.5 Mrad-irradiated samples (Figure 1c) shows the presence of eight compounds not detected in the corresponding nonirradiated sample. Of these eight compounds we have identified, by means of mass spectrometry, four are known to be present in the volatiles from nonirradiated strawberries, but whose concentrations have been increased by irradiation. These are: isobutyric acid (X1); acetic acid (X4); butyric acid (X6); and benzyl acetate (X7). One new compound, however, is present which has not previously been reported as being present in the volatiles from nonirradiated strawberries. We have tentatively identified the compound by mass spectrometry to be methoxyacetaldehyde (X2).

The concentration of volatiles obtained from the relatively small 10-g sample of strawberries would not reveal all of the constituents present. In fact, previously reported work on coupled gas chromatography–mass spectrometry of nonirradiated strawberries used 25 kg (Tressel *et al.*, 1969) and in another 10 tons of vapor condensate were processed (McFadden *et al.*, 1965). However, it is of particular interest that our investigation still revealed eight new peaks in the irradiated strawberries not present in the corresponding nonirradiated samples. Further, the mass spectrometry resolved questions regarding the chemical identity of apparently similar peaks in the gas chromatograms.

Carbonyl Yields. When the dialysates prepared from unirradiated, fresh whole strawberries were treated with 2,4-DNP according to our previously described procedure (Sanders and Schubert, 1971), the visible absorption spectrum has no real maximum in the 430- or 515-nm region as do normal carbonyls. However, when dialysates from irradiated strawberries were treated with 2,4-DNP and the difference spectrum was obtained by using the 2,4-DNP-treated unirradiated strawberry dialysate as the blank, typical carbonyl spectra were obtained (Figure 2). An approximate estimate of the apparent carbonyls in unirradiated strawberries gave values, depending on the batch or variety of strawberry, in the range of 2–3 μmol/g—a level which corresponds to that produced by high levels (1–3 Mrad) of irradiation. When the dialysates of unirradiated strawberries were autoclaved, the resulting 2,4-DNP spectrum resembles that of typical carbonyls. This suggests that autoclaving may destroy or volatilize interfering substances. However, the apparent carbonyl level in unirradiated strawberries is not appreciably changed (±20%) by autoclaving.

The production of carbonyls in strawberries increased linearly with radiation dose up to 1 Mrad. The total carbonyls produced by irradiation, expressed as μmol/g of fresh strawberries determined from assays on dialysate preparations for six irradiation doses ranging from 200 to 3000 krad, is shown in Figure 3. Considering the high background of naturally occurring carbonyls in strawberries, the results are reasonably consistent and reproducible. Similar results were obtained when the carbonyl analyses were carried out on supernatants obtained after

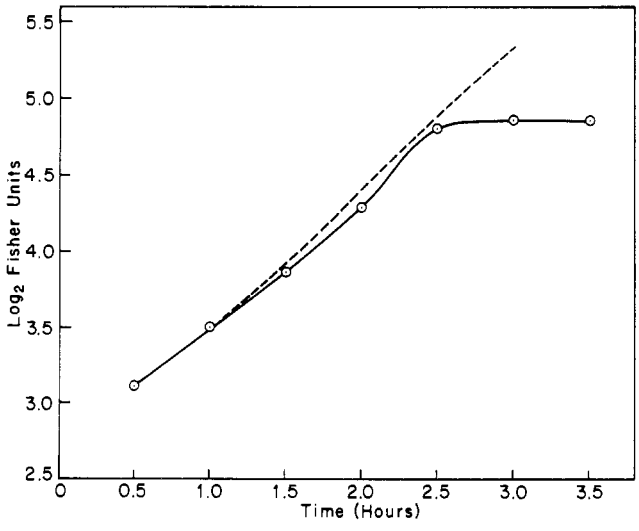


Figure 4. Growth of *Salmonella typhimurium* LT2 in the presence of dialysates (2.5 ml incorporated in growth medium giving total value of 5.0 ml) prepared from irradiated fresh whole strawberries. -O-, glucose control; - - - - -, unirradiated and irradiated (200, 600, and 800 Krad) with and without autoclaving. See text for further experimental details.

centrifugation of the homogenates. However, addition of the 2,4-DNP caused coagulation, which complicated the spectra.

The calculation of total carbonyl concentration is given by the expression

$$C = 24A/\epsilon \quad (1)$$

where C is the concentration of carbonyls in μmol/g of fresh weight, 24 is a dilution factor, A is the measured absorbance from the difference spectra at 430 nm, and ϵ is the molar absorptivity of the 2,4-dinitrophenylhydrazone anions. For these calculations, ϵ is taken as 18×10^3, which is an average value found (Sanders and Schubert, 1971) for a large number of model carbonyl 2,4-dinitrophenylhydrazone anions.

The carbonyls present in the dialysate are quite stable, as shown by the fact that after 20 days storage in the refrigerator the carbonyl content had decreased only 16%. In a separate experiment, strawberries were irradiated at three doses (0.2, 0.5, and 1.5 Mrad) and divided into four portions. Portions were then dialyzed and analyzed for carbonyls 1, 4, 7, and 10 days after irradiation. No change in *apparent* carbonyl levels was observed for 0.2 and 0.5 Mrad doses. However, the absorption at 500 nm increased by a factor of 2 during this time, indicating the formation of a new substance. For strawberries irradiated with 1.5 Mrad, the apparent carbonyls decreased about 30%, while a similar but smaller increase was noticed at 500 nm. The absence of any absorption peak at 550 nm for the visible spectra of 2,4-DNP-treated strawberry dialysates (Figure 2) proves that no glyoxal (<2 μg/ml) is present in either unirradiated and irradiated strawberries.

The radiolytic yield of carbonyls is expressed by the initial G value, where G represents the molecules or ions reacted or formed per 100 eV of absorbed energy. In chemical terms this corresponds to μmol/l. of product per krad. From the slope of the linear part of Figure 3, the initial G(carbonyl) yield in irradiated, fresh whole strawberries was calculated and found to be 2.2.

The effect of autoclaving in open vessels on carbonyl levels in dialysates was investigated. No significant decrease in carbonyl levels was observed for dialysates from 0.2 and 0.5 Mrad-irradiated strawberries, while a 50% decrease was noted for 1.5, 2.0, and 3.0 Mrad dialysates. For reasons not entirely clear, autoclaving in sealed vessels produces a 30% decrease in apparent carbonyl levels for 1.5 Mrad dialysates (30%) and also for 0.2 and 0.5 Mrad dialysates (50%).

The decrease in carbonyl concentration upon autoclaving was caused presumably by the loss of carbonyls through volatilization or chemical reaction. Further exploration of this point revealed that at relatively low doses (200–500 krad) the majority of the carbonyls were not volatile. Distillation of either dialysates or untreated homogenates was conducted at 50° (1 mm) into a series of three traps containing ice water, Dry Ice–methanol, and liquid nitrogen, respectively. Under these conditions no carbonyls could be detected in distillates of 0.2 and 0.5 Mrad-irradiated strawberries. Recoveries from spiked samples averaged 92%. Moreover, when dialysates were distilled, the residues were found to contain all the radiolytically formed carbonyls. Distillates of 1.5 Mrad-irradiated strawberries, however, contained about 60% of the radiolytically produced carbonyls.

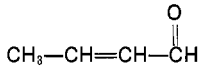
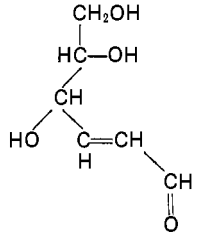
The carbonyl compounds produced upon radiolysis of dilute (5.8×10^{-2} *M*) aqueous solutions of sugars appear to be nonvolatile dicarbonyl sugars in equilibrium with α,β-unsaturated carbonyl sugars. The concentration of these dicarbonyl sugars is not only unaffected by autoclaving, but the biological activity increases severalfold because thermal dehydration and/or enolization increases the concentration of the α,β-unsaturated carbonyls (Schubert and Sanders, 1971). Therefore, it would appear that little or no enolizable or hydroxylated α,β-unsaturated carbonyl compounds are produced upon irradiation of fresh strawberries. It is known, however, that small amounts of the nonhydroxylated α,β-unsaturated carbonyl, *trans*-2-hexenal, occur naturally in strawberries (Gaunt *et al.*, 1971).

Spectral evidence for the absence of enolizable α,β-unsaturated carbonyl compounds is based on the fact that no difference is found between the ultraviolet spectra of dialysates from unirradiated and irradiated strawberries, in contrast to the intense and pH-dependent ultraviolet absorption spectra (sharp peak at about 275 nm) produced by irradiation of aqueous sugar solutions (Schubert and Sanders, 1971). The ultraviolet absorption of unirradiated or irradiated strawberry dialysates taken against water possesses a single broad absorption peak in the 265–275 nm region. Further, the absorption spectra of strawberry dialysates in the 225-nm region rise without apparent limit in contrast to irradiated sugar solution, which possesses a minimum at *ca.* 225 nm. The former phenomenon precludes spectral detection of compounds like *trans*-2-hexenal, which absorbs in the 210–250 nm region. However, the production of significant levels of such compounds by irradiation is excluded by the biological tests described later.

The apparent absence of the highly reactive α,β-unsaturated carbonyl compounds in irradiated strawberries could conceivably be due to destruction by components in the strawberries released upon homogenization. However, the lack of appreciable concentration of amino acids and sulfhydryl groups capable of reacting with the α-β-unsaturated carbonyls would suggest otherwise. Accordingly, we investigated the possibility that strawberry homogenates would, indeed, destroy α,β-unsaturated carbonyls. Homogenates containing 2% by weight of fresh strawberries were prepared from fresh whole strawberries less calyx in the presence of water or aqueous solutions containing different but known α,β-unsaturated carbonyl compounds, namely crotonaldehyde, irradiated sucrose solution, and an autoclaved solution of 2-deoxy-D-glucose, which contains a mixture of *cis*- and *trans*-D-*erythro*-hex-2-enoses (Sanders and Schubert, 1972). The homogenates were centrifuged in the cold (5°) for 20 min at g = 1800.

Ultraviolet difference spectra were taken of the three supernatants containing the α,β-unsaturated carbonyls.

Table III. Test for Interaction of α,β-Unsaturated Carbonyls upon Addition to Fresh Whole Strawberries Followed by Homogenization

Substance	Structure	Concn (μmol/ml)	λ_{max} (nm)	Absorbance, A: Pure substance	Absorbance, A: In presence of strawberries[a]
Crotonaldehyde		0.06	222	1.06	0.85
Autoclaved 2-deoxy-glucose		0.12	215	1.45	1.45
Irradiated (400 krad) sucrose		5.6[b]	265	0.99	1.08

[a] Spectra of the solutions prepared from strawberries and aqueous carbonyl solutions were obtained by blanking out the strawberry absorbance with an aqueous strawberry homogenate. Values are corrected for dilution and light path. [b] This value represents total carbonyls, the majority of which are not biologically active or uv absorbing.

Table IV. Cytogenetic[a] Effect of Irradiated and Nonirradiated Strawberry Dialysates[b] *in Vitro*

Strawberry dialysate	No. of aberrations[c]: No. of damaged cells: B_1	B_2	B_3	C_u	Extensively[d] damaged cells: MA	Pulv	Number of cells scored	Total cells with aberrations: Chromatid ($B_1+B_2+B_3$): No.	%	Chromosome (C_u + MA + P): No.	%	Aberration-free cells: No.	%
Control, saline													
0.5 ml	11/9	0	1/1	0	0	0	50	10	20	0	0	40	80
0.25 ml	8/7	0	1/1	0	0	0	50	8	16	0	0	42	84
X_0 (nonirradiated)													
0.5 ml	9/9	5/3	1/1	4/4	1	0	50	13	26	5	10	32	64
0.25 ml	11/8	0	1/1	3/3	0	0	50	9	18	3	6	38	76
$X_{0.2}$ (irradiated, 200 krad), Sample A													
0.5 ml	14/7	3/3	3/3	5/4	1	0	50	13	26	5	10	32	64
0.25 ml	10/7	0	4/4	4/4	0	0	50	11	22	4	8	35	70
$X_{0.2}$ (irradiated, 200 krad), Sample B													
0.5 ml	8/5	0	3/3	2/2	0	0	50	8	16	2	4	40	80
0.25 ml	7/7	0	3/3	2/2	0	0	50	10	20	2	4	38	76
$X_{0.5}$ (irradiated, 500 krad)													
0.5 ml	12/9	3/0	4/3	14/11	1	0	50	12	24	12	24	26	52
0.25 ml	12/9	3/2	2/1	7/7	0	0	50	12	24	7	14	31	62
$X_{1.5}$ (irradiated, 1.5 Mrad)													
0.5 ml	22/12	0	5/4	9/8	0	0	50	16	32	8	16	26	52
0.25 ml	13/11	1/1	6/6	4/4	0	0	50	18	36	4	8	28	56

[a] Human leukocyte culture–48-hr culture followed by incorporation of 0.5 ml (contains extractant from 165 mg of fresh strawberries) or 0.25 ml of dialysate in the culture medium (total volume = 5.0 ml) and incubation for an additional 24 hr. [b] Dialysates prepared from whole fresh strawberries as described in text. [c] B_1 = chromatid gap. B_2 = chromatid break in nonalignment. B_3 = isochromatid break. C_u = chromosome fragments; dicentric, tricentric, or ring chromosome. [d] MA = multiple aberrations (>10 per cell). Pulv = pulverized chromosomes.

The spectra were then compared to spectra of the pure aqueous carbonyl solutions which had been used to prepare the homogenates (Table III). For autoclaved 2-deoxy-D-glucose and irradiated sucrose, no decrease in the intensity of the ultraviolet absorption maximum was noted. Although a small decrease for crotonaldehyde was observed, we attach no significance to it due to the high background in that region of the ultraviolet. Consequently, these results furnish additional evidence for the absence or, at most, very low levels of α,β-unsaturated carbonyls in irradiated or unirradiated strawberries.

Bacterial Growth. The antibacterial action of unautoclaved and autoclaved dialysates prepared from the irradiated (200, 600, and 800 krad) fresh whole strawberries was measured using the growth of *Salmonella typhimurium* LT2. We incorporated 2.5 ml of dialysate in the growth medium (total volume = 5.0 ml). Autoclaving of the dialysates (15 lb/in.^{2} for 20 min at 121°) caused their color to change from red/pink to orange/yellow. The medium containing the dialysates became very dark after ½ hr of incubation, which required appropriate blanks to be used for the photometric readings. No difference in the growth rate of the bacteria was found between unirradiated or irradiated strawberry dialysates and neither did autoclaving of the dialysates have any observable effect (Figure 4). The medium containing strawberry dialysates overgrew, as expected, because they increased the glucose content of the medium.

Table V. Cytogenetic[a] Effect of Autoclaved Irradiated and Nonirradiated Strawberry Dialysates[b] *in Vitro*

Strawberry dialysate	No. of aberrations[c] No. of damaged cells				Extensively[d] damaged cells		Number of cells scored	Total cells with aberrations: Chromatid ($B_1 + B_2 + B_3$)		Total cells with aberrations: Chromosome (C_u + MA + P)		Aberration-free cells	
	B_1	B_2	B_3	C_u	MA	Pulv		No.	%	No.	%	No.	%
Control, saline													
0.5 ml	8/6	0	2/2	2/2	0	0	50	8	16	2	4	40	80
X_0 (nonirradiated)													
Autoclaved open[e]	9/7	0	1/1	5/5	0	0	50	8	16	5	10	37	74
Autoclaved sealed[f]	25/17	6/2	6/4	3/3	0	0	50	23	46	3	6	24	48
$X_{0.2}$ (irradiated, 200 krad)													
Autoclaved open[e]	18/7	5/3	4/4	5/5	0	0	50	14	28	5	10	31	62
Autoclaved sealed[f]	20/13	1/1	4/4	4/4	0	0	50	18	36	4	8	28	56
$X_{0.5}$ (irradiated, 500 krad)													
Autoclaved open[e]	21/15	2/1	5/5	0	0	0	50	21	42	0	0	29	58
Autoclaved sealed[f]	13/8	2/1	2/1	6/5	0	0	50	10	20	5	10	35	70
$X_{1.5}$ (irradiated, 1.5 Mrad)													
Autoclaved open[e]	18/12	4/1	8/6	9/9	0	0	50	19	38	9	18	22	44
Autoclaved sealed[f]	13/8	5/4	7/5	7/6	0	0	50	17	34	6	12	27	54

[a] Human leukocyte culture–48-hr culture followed by incorporation of 0.5 ml (contains extractant from 165 mg of fresh strawberries) of dialysate in the culture medium (total volume = 5.0 ml) and incubation for an additional 24 hr. [b] Dialysates prepared from whole fresh strawberries as described in text. [c] B_1 = chromatid gap. B_2 = chromatid break in nonalignment. B_3 = isochromatid break. C_u = chromosome fragments; dicentric, tricentric, or ring chromosome. [d] MA = multiple aberrations (>10 per cell). Pulv = pulverized chromosomes. [e] Autoclaved at 15 psi (121°) for 20 min in flasks loosely stoppered with cotton plugs. [f] Autoclaved at 15 psi (121°) for 20 min in sealed ampoules.

Table VI. Cytogenetic[a] Effect of Distillates from Irradiated and Nonirradiated Strawberry Dialysates[b] and Homogenates[b] *in Vitro*

Strawberry distillates	No. of aberrations[c] No. of damaged cells				Extensively[d] damaged cells		Number of cells scored	Total cells with aberrations: Chromatid ($B_1 + B_2 + B_3$)		Total cells with aberrations: Chromosome (C_u + MA + P)		Aberration-free cells	
	B_1	B_2	B_3	C_u	MA	Pulv		No.	%	No.	%	No.	%
Control, saline													
0.5 ml	4/4	0	1/1	2/2	0	0	50	5	10	2	4	43	86
X (nonirradiated)													
Distillate from dialysate	7/6	1/1	3/3	2/2	0	0	50	10	20	2	4	38	76
Distillate from homogenate	12/11	1/1	0	4/4	0	0	50	12	24	4	8	34	68
Residue from dialysate[e]	11/10	2/2	5/4	3/3	0	0	50	16	32	3	6	31	62
X (irradiated, 200 krad)													
Distillate from dialysate	12/9	2/2	1/1	1/1	0	0	50	12	24	1	2	37	74
Distillate from homogenate	9/8	2/2	0	3/3	0	0	50	10	20	3	6	37	74
Residue from dialysate[e]	16/12	1/1	0	2/1	0	0	50	13	26	1	2	36	72
X (irradiated, 50 krad)													
Distillate from dialysate	10/7	2/2	4/4	6/6	0	0	50	13	26	6	12	31	62
Residue from dialysate[e]	11/9	3/3	0	4/4	0	0	50	12	24	4	8	34	68
X (irradiated, 1.5 Mrad)													
Distillate from dialysate	7/6	0	3/3	0	0	0	50	9	18	0	0	41	82
Distillate from homogenate	12/7	3/3	2/2	3/3	0	0	50	12	24	3	6	35	70
Residue from dialysate[e]	8/6	0	2/1	6/5	0	0	50	7	14	5	10	38	76

[a] Human leukocyte culture–48-hr culture followed by incorporation of 0.5 ml (contains extractant from 165 mg of fresh strawberries) of distillate in the culture medium (total volume = 5.0 ml) and incubation for an additional 24 hr. [b] Dialysates and homogenates prepared from whole fresh strawberries as described in text. [c] B_1 = chromatid gap. B_2 = chromatid break in nonalignment. B_3 = isochromatid break. C_u = chromosome fragments; dicentric, tricentric, or ring chromosome. [d] MA = multiple aberrations (>10 per cell). Pulv = pulverized chromosomes. [e] Residue diluted to original volume before incubation.

Each milliliter of dialysate represented the extractant from 330 mg of the original fresh whole strawberries. Therefore, a milliliter of growth medium contained extractant from 165 mg of the original fresh whole strawberries. At the highest radiation dose tested, each milliliter of growth medium contains the equivalent of about 0.3 μmol of radiolytically-produced carbonyls (Figure 2). This corresponds to about 0.5×10^{-8} μmol/cell—an amount which would produce measurable inhibition only if all the carbonyls present were as inhibitory as α,β-unsaturated carbonyl compounds (Schubert and Sanders, 1971).

The effect on bacterial growth of dialysates prepared from strawberries receiving 1.5 Mrad was tested. From 0.5 to 2.3 ml of dialysate was incorporated in the growth medium. Again, no significant inhibitory action was observed.

Cytogenetics. *In Vitro.* Unirradiated and irradiated dialysates and distillates, prepared from fresh whole California strawberries, were incorporated in the human lymphocyte culture. In Table IV are tabulated the cytogenetic results obtained for irradiated strawberry dialysates (0.2, 0.5, and 1.5 Mrad). No statistically significant difference ($p = 0.01$ using the Yates correction) between unirradiated and the irradiated strawberries was noted. However, at doses of 0.5 and 1.5 Mrad, it is possible that an increase in clastogenic substances produced by irradiation may

Table VII. Cytogenetic[a] Effect of Irradiated and Nonirradiated Strawberry Puree on Mice *in Vivo*[b]

Strawberry puree	Post-intubation, days	No. of aberrations: No. of damaged cells: Chromatid B_1	Chromatid B_2	Chromatid B_3	Chromosome C_u	Chromosome C_s	No. of cells scored/no. of animals	Total cells with aberrations: Chromatid No.	Chromatid %	Chromosome No.	Chromosome %	Aberration-free cells: No.	%
Saline control	1	0	0	0	0	0	14/3	0	0	0	0	14	100
	4	1/1	0	0	0	0	28/4	1	4	0	0	27	96
	7	1/1	0	0	0	0	130/7	1	1	0	0	129	99
Nonirradiated	1	0	0	1/1	0	0	54/3	1	2	0	0	53	99
	4	0	0	0	0	0	98/5	0	0	0	0	98	100
	7	0	0	0	0	1/1	99/5	0	0	1	1	98	99
Irradiated (1.5 Mrad)	1	1/1	0	0	0	0	17/3	1	6	0	0	16	94
	4	0	0	0	0	1/1	43/4	0	0	1	2	42	98
	7	3/3	0	0	0	2/2	171/7	3	2	2	1	166	97

[a] The cytogenetic analyses were carried out on bone marrow cells obtained by aspiration from the femurs as discussed in the text. [b] The 11-week-old Swiss-Webster mice were given 0.5 cm³ of strawberry puree three times a day by intubation for 5 days. Each group consisted of seven animals, at least three of which were used to supply bone marrow cells for a given day postintubation. The scoring was the same as that on Table IV except for the addition of C_s, which represents abnormal chromosome or marked chromosome.

Table VIII. Cytogenetic Effects of Irradiated and Nonirradiated Strawberry Puree on Rats *in Vivo*[a]

Strawberry puree	Post-intubation, days	No. of aberrations: No. of damaged cells: Chromatid B_1	Chromatid B_2	Chromatid B_3	Chromosome C_u	Chromosome C_s	No. of cells scored/no. of animals	Total cells with aberrations: Chromatid No.	Chromatid %	Chromosome No.	Chromosome %	Aberration-free cells: No.	%
Nonirradiated	1	0	0	0	0	0	55/3	0	0	0	0	55	100
	3												
	4	0	0	0	0	0	20/1	0	0	0	0	20	100
Irradiated (150 Krad)	1	0	0	0	0	0	60/3	0	0	0	0	60	100
	3	1/1	0	0	0	0	40/2	1	3	0	0	39	97
	4	0	0	0	0	0	20/1	0	0	0	0	20	100
Irradiated (300 Krad)	1	4/4	0	0	1/1	0	60/3	4	7	1	2	55	92
	3	0	0	0	0	0	35/2	0	0	0	0	35	100
	4	0	0	0	0	0	20/1	0	0	0	0	20	100

[a] The rats used were 250–300-g Sprague–Dawley males. They were given 4.0 cm³ of strawberry puree three times a day for 5 days. All other details of the experiment were the same as those described in Table V.

occur, but it would require a much larger sample size to verify, particularly in view of the high background of unirradiated strawberry preparations.

Table V summarizes cytogenetic results when strawberry dialysates at the same three radiation doses were autoclaved following irradiation. Autoclaving was conducted in flasks sealed only with cotton plugs, *i.e.*, under conditions where volatile constituents would be removed, or in sealed glass ampoules. No significant differences ($p = 0.01$) for chromosome aberrations between irradiated and unirradiated samples were observed. A statistically significant ($p = 0.01$) difference in the number of chromatid aberrations was observed in those samples which were irradiated at 0.5 and 1.5 Mrad in unsealed flasks. Similarly, a significant increase in chromatid aberrations was observed in nonirradiated samples autoclaved in a sealed ampoule as compared with a sample autoclaved in an unsealed flask. These results suggest that the autoclaving of irradiated strawberry dialysates in open flasks at 0.5 and 1.5 Mrad produces clastogenic substances acting on the G_2 phase of cell division. However, these effects appear minor, and in any case the experiments must be repeated with larger samples. Consequently, further evaluations at this time are not warranted.

We also tested distillates and residues from nonautoclaved dialysates prepared from irradiated whole strawberries. The results, summarized in Table VI, show that neither the distillates nor the residues in irradiated samples differ significantly ($p = 0.01$) from the corresponding nonirradiated samples. These negative findings confirm those found on the strawberry dialysates reported in Table IV.

Cytogenetics. *In Vivo.* Irradiated strawberries (1.5 Mrad) were crushed and given to mice for several days by intubation, as described in the Experimental Section. The results shown in Table VII reveal the absence of clastogenic effects by unirradiated or irradiated strawberries.

Another series of experiments utilizing the freeze-dried strawberry powders (0, 150, and 300 krad) given by intubation was undertaken using rats as the test animal. As the results summarized in Table VIII show, no clastogenic effects were found.

In summary, we have shown that as is the case with aqueous sugar solutions (Schubert and Sanders, 1971; Schubert *et al.*, 1971), γ-irradiation of whole fresh strawberries also produces carbonyl compounds. However, the nature of these compounds appears to be different than the dicarbonyl sugars produced from simple aqueous monosaccharide or disaccharide solutions. This is shown by the lack of significant biological effects of irradiated strawberry dialysates, which contain radiolytically produced carbonyls, and by the absence of the ultraviolet ab-

sorbing material present in irradiated dilute aqueous solution of sugars.

ACKNOWLEDGMENT

The authors are grateful for the technical assistance by James L. Meade and James Stragand and Julia Chen on the carbonyl assays; to E. R. White, L. E. Becker, and E. Nordstrand on the gas chromatographic analyses; to M. Clendening for the bacterial assays; and to Hazel S. Li and Virginia M. Sanders for the cytogenetic preparations.

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Received for review October 12, 1972. Accepted April 6, 1973. This research was supported by the Division of Biology and Medicine, U.S. Atomic Energy AT(11-1)-3430.

Relationship between Tannin Levels, Rat Growth, and Distribution of Proteins in Sorghum

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Three high tannin varieties of sorghum gave significantly lower growth responses in weanling rats when compared with three low tannin varieties. Whole kernel and hand-separated endosperms were fractionated by the Landry and Moureaux method to yield five soluble fractions. Nitrogen recoveries ranged from 83 to 96%. Distribution of proteins among the five fractions was distinctly different in the high tannin and the low tannin sorghums, especially in fractions I and V.

The discovery that the opaque-2 gene in corn improves protein quality (Mertz *et al.*, 1964) has stimulated considerable interest among breeders, agronomists, nutritionists, and biochemists in improvement of protein quality and quantity of other cereal grains. Sorghum grain ranks fifth in acreage of crops of the world and forms the basic food in many parts of Africa and Asia. In order to improve the quality of sorghum grain, it is desirable to separate the proteins of sorghum and study the individual fractions in detail. Published literature has been very limited because of the insoluble characteristics of sorghum grain proteins. The procedure that has been employed for the fractionation of sorghum proteins by other workers (Haikerwal and Mathieson, 1971; Jones and Beckwith, 1970; Skoch *et al.*, 1970; Virupaksha and Sastry, 1968) is based on the classical procedure of Osborne and Mendel (1914). Some of the problems that have been associated with this procedure have been reported by the above workers, such as low nitrogen recoveries (Skoch *et al.*, 1970), and the inability to work with the alcohol fractions (Jones and Beckwith, 1970) and with the glutelin fractions (Haikerwal and Mathieson, 1971) due to the gelling of these fractions. Thus, Osborne and Mendel's (1914) procedure and

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