Hydroponic Growth of Crops in Solutions Saturated with [^{14}C]Benzo[*a*]pyrene

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The translocation of [^{14}C]benzo[*a*]pyrene has been studied by growing crops hydroponically in nutrient solutions saturated with this compound. The crops studied in this fashion were green beans, cantaloupes, and cottonseeds. Carbon-14 labeled benzo[*a*]pyrene was used to achieve a high level of sensitivity and primary analyses were made by conventional liquid scintillation counting techniques. From this work, it has been concluded that benzo[*a*]pyrene did not translocate or concentrate in the crops tested. None was detected at a sensitivity of 3 parts per billion. Radioactivity corresponding to a maximum of 8 parts per billion was found in some composite bean leaf and cottonseed samples, but following extraction, column chromatography, UV fluorescence, and reassay, no (<3 ppb) benzo[*a*]pyrene was found. A minor degree of degradation of [^{14}C]benzo[*a*]pyrene to volatiles followed by irreversible deposition into the plant cuticle provided the most probable explanation for the traces of radioactivity found.

Concerns about the possible entry of carcinogens into the food chain have been ever increasing, due in part to the recent identification of more of such species and to the levels and variety of pollution in our environment. Efforts to identify, control, and eliminate such health hazards form part of the mandate of authorized regulatory agencies. For example, the Environmental Protection Agency evaluates the impact of new and existing industrial products and wastes on our environment and the Food and Drug Administration regulates products intended for use in food-related applications. When such products are petroleum derived, polynuclear aromatic hydrocarbons are of particular interest since, as reported by Hartwell, some have been found to possess carcinogenic activity. While direct exposure to such hazards are an obvious concern, possible indirect contamination of food such as agricultural products cannot be overlooked. Whereas polynuclear aromatic hydrocarbon analyses of a variety of foods have been reported, as summarized in a review of this subject by Howard and Fazio (1969), the investigator has been left to speculate on the origin of such materials where positive results were obtained.

In this study, an attempt was made to evaluate the fate of a polynuclear aromatic hydrocarbon under severe and exaggerated experimental conditions. Green beans, cantaloupes, and cotton were grown hydroponically in nutrient-containing aqueous solutions saturated with carbon-14 labeled benzo[*a*]pyrene, a known carcinogen. The nutrient blend was not identified but was supplied by the Agricultural Department of Rutgers University. It was felt that the hydroponic experiments would provide a much better indication of the true migration into plants than migration from soil. Experiments in soil offer opportunity for bacterial and chemical attack. Also it was expected that migration would generally follow a water solubility mechanism. The plants were selected because of different growing times involved: 6, 12, and 22 weeks for green beans, cantaloupes, and cotton, respectively, as well as the great differences in these plant types.

EXPERIMENTAL SECTION

The nutrient solutions for hydroponic growth were first saturated with [9-^{14}C]benzo[*a*]pyrene, whose sp act. was 8.83 mCi/g. In two publications, Davis et al. (1942) reported a water solubility of 4 parts per billion (ppb) using a nephelometric method. To insure saturation, twice this amount was added. The expected saturation level was confirmed by radioassay and UV fluorescence analyses at the beginning and end of the experiments.

The labeled benzo[*a*]pyrene was obtained from New England Nuclear Corp. It was repurified by column chromatography over 60–80 mesh magnesia previously dried at 110 °C. After prewetting the column with heptane, some impurities were eluted with isooctane and the benzo[*a*]pyrene was recovered with benzene. About 90% was recovered having a chemical purity of about 99% based on UV fluorescence.

Two green bean plants were grown in this medium, three plants for cantaloupes and six plants for cotton. Control plants were also grown hydroponically for comparison purposes. Opaque plastic containers, partially filled with sterilized pebbles were used to support the plants during growth. To confine the radioactivity, the plants were grown in a laboratory hood accessible to light. Make up water was added almost daily to maintain initial levels. At maturity, the fruit of each of the plants were harvested for analysis. In addition, stems and leaves from the green bean plants were analyzed.

Except for the cottonseeds which were analyzed directly, the others were first dehydrated into volatiles and dried plant tissue in the following manner. Green bean plant tissue was dehydrated at 120 °C under a stream of nitrogen for approximately 4 h. These conditions were found to remove volatiles without charring the tissue. Dehydration of cantaloupe pulp was carried out in a rotary evaporator at 90–95 °C and 3–5 mmHg pressure for about approximately 6 h. This slower and less severe approach was necessary for tissue of higher sugar content and, therefore, more susceptible to charring.

Volatiles were collected in a cold trap but were not individually assayed except to confirm negligible activity for disposal purposes. As discussed later, [^{14}C]benzo[*a*]pyrene was not distilled under these experimental conditions. Dried tissue was then ground to powder and stored in vials prior to analysis. This approach of sample concentration resulted in at least a fivefold increase in sensitivity.

About 100 mg of dehydrated plant tissue could be and was combined in a tube furnace in a manner similar to conventional elemental analysis for carbon. The essential difference was that (radioactive) CO_2 produced was collected in a liquid scintillation counting vial containing hyamine. After combustion was complete, the counting vial was filled with scintillator solution comprising toluene

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Table I. Volatility of [^{14}C]Benzo[a]pyrene during Dehydration of Plant Tissue

Plant tissue	Radioactivity, mCi: Added/g	Radioactivity, mCi: Found in volatiles/g	% activity in volatiles
Bean fruit	0.88×10^{-4}	1.4×10^{-8}	0.02
Bean leaf	0.88×10^{-4}	4.8×10^{-8}	0.05
Bean stem	0.88×10^{-4}	2.6×10^{-8}	0.03
Cantaloupe pulp	0.88×10^{-4}	1.4×10^{-8}	0.02

as the solvent and PPO (2,5-diphenyloxazole) and dimethyl POPOP {1,4-bis[2(4-methyl-5-phenyloxazolyl)]benzene} as phosphors.

The vials were then assayed in triplicate for 40-min intervals on a Packard Tri-Carb Liquid Scintillation Spectrometer. Counting efficiency was determined by internal standardization using [^{14}C]toluene and averaged 45%. Since background measurements in this scintillating medium averaged about 30 counts/min, this corresponded to about 3×10^{-8} mCi of radioactivity, equivalent to about 3×10^{-9} g of [^{14}C]benzo[a]pyrene.

Using counting statistics, about 3 counts/min above background was necessary to exceed two standard deviations for a nominal dehydrated sample size of 100 mg. This was equivalent to 30 counts min^{-1} g^{-1} of sample or about 3×10^{-9} g of [^{14}C]benzo[a]pyrene. Therefore, radioactivity below this level (i.e., 3 parts per billion) was judged to be statistically insignificant.

The recoveries of carbon-14 after combustion and collection in the hyamine cocktail were consistently better than 98% using a labeled plastic film obtained from New England Nuclear Corp. and using plant tissue previously spiked with [^{14}C]benzo[a]pyrene.

In order to provide for instances where radioactivity would be measured in plant tissue, it was necessary to develop separations and analysis techniques to determine whether benzo[a]pyrene was present. This was accomplished as follows.

The plant tissue to be examined was extracted with chloroform in a blender (see Scheme I). In the cases of green beans and cantaloupes, the samples were first frozen to insure rupture of cell structures and to facilitate extraction of benzo[a]pyrene. The extracts were chromatographed on silica gel columns, and the eluates containing radioactivity were partitioned between cyclohexane and nitromethane. The nitromethane solutions were then chromatographed on silica gel columns previously impregnated with nitromethane. The final eluates were examined by UV fluorescence and assayed.

RESULTS AND DISCUSSION

As noted, [^{14}C]benzo[a]pyrene did not volatilize under dehydration conditions. This was demonstrated by adding [^{14}C]benzo[a]pyrene to unexposed samples of plant tissue and assaying the volatiles collected in a 3:1 toluene–methanol solution containing the phosphors identified earlier. Counting efficiency averaged about 45%, and the data obtained are shown in Table I.

The results of plant tissue are presented in Tables II, III, and IV for green beans, cantaloupes, and cotton, respectively. Data obtained for control plants are presented along with those for exposed plants for comparative purposes. If any net activity remained, the maximum possible concentrations of [^{14}C]benzo[a]pyrene were computed based on its known sp act. of 8.83 mCi/g.

As seen in Table II, the green bean stems showed no net radioactivity; and while the bean tissue showed net activity corresponding to a maximum of 2 ppb of [^{14}C]benzo[a]pyrene, this result was not considered to be significant, given a test sensitivity of about 3 ppb. Because the average values for leaves corresponded to maximum concentrations of 7–8 ppb, further separations and analysis were indicated.

The remaining bean and leaf tissues, about 1:2 parts by weight, were combined to increase the sample size. Whereas the concentration of radioactivity was not increased by this approach, the absolute amount available for extraction was greater, thereby enhancing detectability. This sample was then subjected to the separations scheme

Table II. Radioassay of Green Bean Tissue[a]

Leaf tissue	Wt % dehydrated tissue	Sp act., mCi × 10^9/g: Dehydrated tissue: Control	Sp act., mCi × 10^9/g: Dehydrated tissue: Sample	Sp act., mCi × 10^9/g: Total tissue: Control	Sp act., mCi × 10^9/g: Total tissue: Sample	Max. ppb [^{14}C]benzo[a]pyrene above av control
1		72	200	8	23	2
2		25	1130	2	131	14
3		28	457	2	53	5
Av	11.6	42	596	4	69	7
Stem tissue						
Lower		100	93	21	19	
Middle		117	29	24	6	
Upper		83	93	17	19	
Av	20.5	100	72	21	15	
Leaf tissue						
1		328	1284	38	149	13
2		121	1121	14	130	11
3		319	241	37	28	
Av	11.6	256	882	30	102	8
Bean tissue						
1		26	217	3	25	2
2		17	191	2	22	2
3		165	243	19	28	2
Av	11.5	69	217	8	25	2
Composite					11[b]	1[b]

[a] At least two assays were made, each counted in triplicate. [b] Composite sample of leaf and bean tissues after separations. No benzo[a]pyrene detected by UV fluorescence.

Scheme I. Separation of Crop Extracts

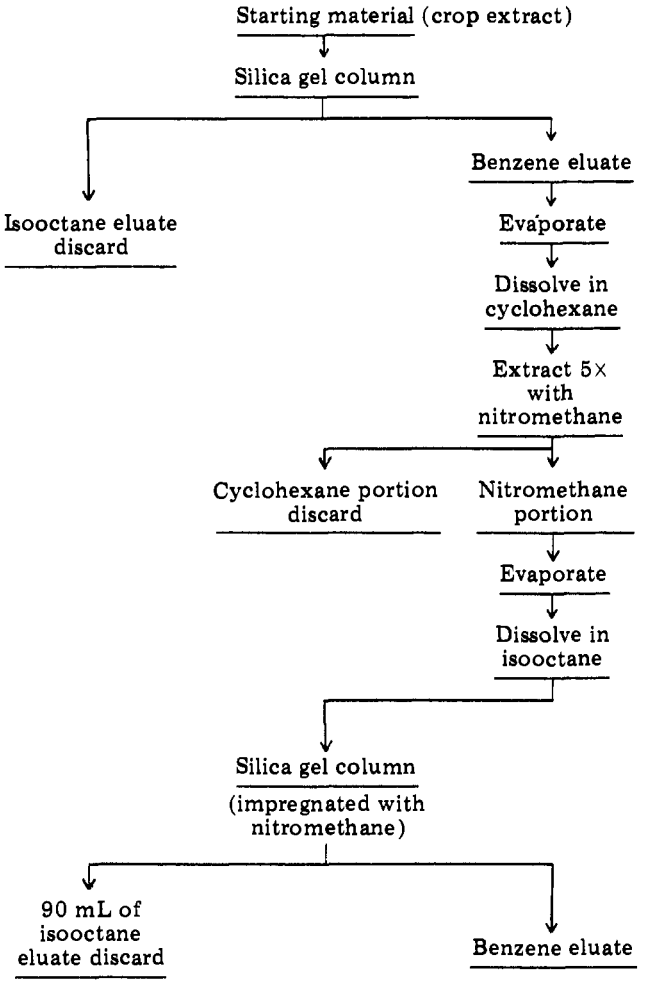

Table III. Radioassay of Cantaloupe Tissue[a]

	Wt % dehydrated tissue	Sp act., mCi × 10⁹/g		Max. ppb [14C]benzo[a]pyrene above av control
		Dehydrated tissue	Total tissue	
Control fruit				
1	7.3	138	10	
2	5.3	237	13	
Av	6.3	188	12	
Exposed fruit				
1	5.1	147	7	--
2	5.0	126	6	--
3	3.8	145	6	--
Av	4.6	139	6	--

[a] At least two assays were made, each counted in triplicate.

described earlier. As shown in Table II, the net activity corresponded to a maximum of 1 ppb but was considered to be within experimental error and therefore not significant. Benzo[a]pyrene could not be detected by UV fluorescence on this concentrate. The applicability of the separations procedure was demonstrated by adding benzo[a]pyrene to plant tissue samples before dehydration and measuring recoveries at the conclusion of the drying and separation phases. These results are presented in Table V. In one separate instance, [14C]benzo[a]pyrene was added to a cottonseed sample. Though the recovery

Table IV. Radioassay of Cottonseeds[a]

	Sp act., mCi × 10⁹/g	Maximum ppb as [14C]benzo[a]pyrene		After separations	
		Gross	Net	Net sp act., mCi × 10⁹/g	Maximum ppb as [14C]benzo[a]pyrene
Control samples					
1	47	5.3			
2	159	18.0			
3	137	15.0			
4	78	8.8			
5	27	3.1			
6	57	6.5			
Av	84 ± 52(σ)	9.5			
Composite sample				15[b]	b
Exposed samples					
1	79	9.0			
2	58	6.5			
3	86	9.8	0.3		
4	76	8.6			
5	159	18.1	8.6		
6	120	13.6	4.1		
Av	96 ± 37(σ)	10.9	1.4		
Composite sample				10[b]	b

[a] At least two assays were made, each counted in triplicate. [b] No benzo[a]pyrene detected by UV fluorescence.

Table V. Recovery of Benzo[a]pyrene from Cottonseed, Cantaloupes, and Green Beans

Crop	Benzo[a]pyrene added, ppb	% recovery by UV fluorescence	% recovery by radioassay
Cantaloupes	10	99	
Green beans	10	98	
Cottonseed	10	65	
Cottonseed[a]	10	48	53

[a] [14C]Benzo[a]pyrene used.

was lower than previously observed, there was good agreement between fluorescence and radioassay data. The high oil content in the cottonseeds contributed interferences which could account for the lower recoveries.

As shown in Table III, no evidence of net radioactivity was detected in the exposed cantaloupe samples. Therefore, further separation and analysis for [14C]-benzo[a]pyrene were not required.

The data in Table IV showed higher levels of radioactivity in control and exposed cotton samples compared with the other plants tested. Greater variability between individual results was also noted. The initial approach to interpretation of these data involved statistical treatment through a t test. Calculations showed a 1% probability that the differences between controls and exposed samples were significant. This suggested that [14C]benzo[a]pyrene migration was not the source of radioactivity. Separations and analysis on composite samples confirmed the absence of benzo[a]pyrene. None was detected by UV fluorescence and no significant radioactivity was found in the concentrates.

Since benzo[a]pyrene was not identified in any of the samples, an alternate explanation would be in order to

account for the traces of radioactivity detected. One possibility could be metabolites of [^{14}C]benzo[*a*]pyrene. However, this would not account for the variable and almost equivalent levels of activity found in many control plant tissues.

A more plausible explanation would be some degradation of the [^{14}C]benzo[*a*]pyrene from the nutrient solutions forming volatile decomposition products that would deposit on the plant cuticles. The waxy cutin would have been sufficiently retentive since tissue dehydration did not result in losses of activity. Since control and test plants were grown in the same laboratory hood area to maintain a similar environment, they would have received similar exposure. This theory was supported by the detection of traces of carbon-14 radioactivity from routine wipe tests made on the hood surfaces at the conclusion of the study.

Decomposition of [^{14}C]benzo[*a*]pyrene was minimal since the nutrient test solutions did not show significant differences in the specific activity (8.83 mCi/g) of the [^{14}C]benzo[*a*]pyrene or in its solution (4 ppb) or total (8 ppb) concentration at the conclusion of the experiments. Significant degradation would have caused at least one of these values to change. The use of opaque plant containers was evidently effective in minimizing any photodecomposition of the benzo[*a*]pyrene.

The higher level of activity found in the cotton plants may therefore be due to greater exposure because of its longer growth period. In contrast, the comparatively low levels in the cantaloupes may be attributed to late appearance of the fruit in its growth cycle. Higher levels in the bean leaves compared with stems and fruit could be explained by their greater surface available for exposure. Assays of these crops, unexposed to [^{14}C]benzo[*a*]pyrene and grown in fields showed no radioactivity above the normal 30 counts/min of background.

The variability of activity found in the samples could be due to a combination of different individual plant growth rates and the extent of which they could have been exposed. Greater variations would be expected with controls because proximity to test plants would influence the extent to which deposition of activity could have occurred.

On the basis of this study, benzo[*a*]pyrene was not found to translocate or concentrate into the three different crops tested under hydroponic growth conditions. In the instance where other parts of the bean plant were examined, the same conclusion could be drawn. The traces of radioactivity found in the plants suggested contamination from volatile degradation products rather than occurrence through a metabolic process.

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Protein Supplementation of Navy Beans with Brazil Nuts

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Rat feeding experiments showed that the protein quality of Navy beans, a poor source of sulfur-containing amino acids, can be largely improved by mixing the beans with defatted Brazil nuts, a rich source of methionine. Compared with a PER (protein efficiency ratio) of 2.50 for casein, the PER for beans was 1.53, and for diets containing bean protein and Brazil nut protein at the ratios 80:20, 90:10, and 95:5, the PER's were 2.42, 2.16, and 1.93, respectively, at 10% total protein content in the diet. A comparison of PER with other protein evaluation measures was made for the diets tested here.

Protein malnutrition is a major health problem in the world. A significant portion of the protein in the diet of many people comes from legumes (Aykroyd and Doughty, 1964). A popular legume, especially in Latin America and South Asia, is the dry seeds of the common bean (*Phaseolus vulgaris*). The bean protein, however, is deficient in the sulfur-containing amino acids, methionine and cysteine (FAO, 1970). On the other hand, the protein of Brazil nuts (*Bertholletia excelsa*) is exceptionally rich, among plant proteins, in sulfur-containing amino acids (FAO, 1970). The objective of this research was to study the supplementary effect of Brazil nut protein on Navy bean protein through rat feeding and a microbiological assay.

MATERIALS AND METHODS

Navy beans, cv Sanilac, grown in Michigan, were obtained from the Michigan Foundation Seed Association. Brazil nut kernels were shipped by air from the Brazilian Institute of Food Technology. These nuts were defatted with hexane. A fire-proof Waring blender was used to grind the kernels with hexane. After blending, the slurry was filtered under reduced pressure and the residue was blended with fresh hexane and filtered five more times. The residue, henceforth referred to as Brazil nut defatted flour (BNDF), was air dried in a ventilated hood for 24 h and stored in a refrigerator. For the feeding studies, the beans were ground in a feed grinder, autoclaved as a thin layer (about 5 mm) in stainless steel pans at 121 °C for 10 min and dried in a ventilated hood. For amino acid

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