gates in feces are probably derived from bile, our results indicate that mice and hamsters may be poor biliary excretors compared with the rat.

Short-term experiments with labeled DDT showed that, except for relatively small amounts of neutral compounds, substantially all urinary metabolites in both species were hydrolyzable conjugates of DDA. DDA has been identified in the urine of all mammalian species examined (Apple, 1968; Hayes, 1965), but this is the first demonstration that in the fresh urine of mouse and hamster nearly all the DDA is conjugated.

The presence of cinnamoylglycine in mouse urine is an interesting species difference although not directly related to DDT metabolism since control mouse urine contained this compound in lesser amounts. Its formation was definitely related to some factor in the Wayne diet since mice on a semisynthetic diet with or without DDT did not excrete it. On the other hand this factor was probably not cinnamic acid itself (or some related compound such as cinnamaldehyde) since addition of sodium cinnamate to the drinking water of animals on the semisynthetic diet did not lead to the excretion of cinnamic acid or cinnamoylglycine in either species.

The most important difference between the species with regard to urinary DDT metabolites was the steadily increasing elimination of DDE in the mouse during the long-term feeding experiments. Apple (1968) orally administered DDT in olive oil to rodents for 5 days and collected urine for 8 days. He reported that mice, in contrast to rats and rabbits, excreted DDE as the principal neutral urinary metabolite although less than 1% of the administered DDT was recovered in this form. Our single dose administrations confirmed this small excretion in the mouse and failed to reveal any in the hamster. After 2 weeks on dietary DDT, however, the mouse was already excreting over 1% of ingested DDT as DDE, and at the termination of the experiments nearly as much DDE as DDA was found.

Biotransformation to DDE is probably not of importance in explaining the acute toxicity of DDT in the mouse since the metabolite is much less toxic than DDT itself (LD_{50} > 1600 mg/kg). Acute toxicity differences in comparison with the hamster may be due to differences in the blood–brain barrier to DDT in these species (Gingell and Wallcave, 1974).

Although the significance of urinary DDE excretion in the mouse is not certain, it is of interest to note that DDE is found in much higher concentrations in the livers of mice fed 250 μg/g of dietary DDT than in corresponding hamster livers (Gingell and Wallcave, 1974). Tomatis *et al.* (1971) reported significant amounts of DDE in mouse kidney but there are no comparative data for the hamster. Furthermore, it has been reported by Tomatis *et al.* (1974) that DDE fed at levels of 250 μg/g in the diet led to a high incidence and early appearance of liver tumors. The authors suggest that the carcinogenic effect of DDT on mouse liver may be a consequence of its conversion to DDE. Our findings of high levels of DDE in mouse liver and urine together with low levels (or none) in the hamster are consistent with such speculations.

ACKNOWLEDGMENT

We wish to thank K. Patil, Health Care Studies, University of Nebraska Medical Center, for statistical analysis of the data.

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Received for review January 18, 1974. Accepted May 20, 1974. [^{14}C]DDT was purchased from funds made available for this purpose by the World Health Organization. Presented in part at the Society of Toxicology, 12th Annual Meeting, New York, N. Y., 1973. This study was supported by National Institutes of Health Contract No. PH-43-68-959.

COMMUNICATIONS

Carotenoids of Yellow and Red Lutescent Tomatoes

The carotenoids of yellow and red lutescent tomato fruits were characterized at several stages of ripeness. The yellow lutescent tomatoes accumulated mostly neurosporene, β-carotene, and lutein during ripening, reaching a final carotenoid level of approximately 40 μg/g dry wt of fruit. The red lutescent tomatoes developed a composition of carotenoids, resembled the normal red tomatoes, and reached a carotenoid level of about 900 μg/g dry wt of fruit. The lutescent gene does not seem to alter drastically the carotenoid composition of the tomato fruits.

The red lutescent tomatoes were mentioned by Mackinney (1958) and used by Simpson and his coworkers (Raymundo *et al.*, 1970; Villegas *et al.*, 1972) in studies of carotenoid biosynthesis. Characteristically, the lutescent gene in tomatoes expresses itself by a premature yellowing of the leaves and an unusual ripening pattern of the fruits. The immature fruits contain much less chlorophyll than normal tomatoes and the fruit turns almost completely white at the mature stage. Several days may elapse before the synthesis of lycopene occurs.

The yellow lutescent tomatoes possess the same characteristics of the red lutescent tomatoes except that the

Table I. Carotenoids of Ripening Yellow Lutescent Tomatoes

Polyenes	Carotenoids, μg/g dry wt		
	I[a]	II	III
Neurosporene	0.7	5.3	18.1
β-Carotene	0.3	4.5	11.8
Lutein	0.3	2.8	7.0
Unknown	0.2	1.5	2.6
Total	1.5	14.1	39.5

[a] Stage I, mature white; II, pale yellow; III, yellow; refers to the ground color of the fruits.

Table II. Carotenoids of Ripening Red Lutescent Tomatoes

Polyenes	Carotenoids, μg/g dry wt					
	I[a]	II	III	IV	V	VI
Phytoene		1.6	36.6	66.5	102.5	122.4
Phytofluene		0.5	19.6	35.8	72.8	74.5
ζ-Carotene	0.1	0.3	11.0	15.6	20.5	22.1
Lycopene	0.5	9.5	103.4	282.7	413.8	619.3
β-Zeacarotene			2.2	3.3	2.5	
δ-Carotene			8.0	10.1	7.3	8.2
β-Carotene	2.3	15.1	26.3	34.1	38.0	40.0
Unknown			1.1	2.2	1.2	2.6
Total	2.9	27.0	207.1	450.3	658.6	889.1

[a] Stage I, white; II, yellow; III, yellow-orange; IV, orange; V, red-orange; VI, red; refers to the ground color of the fruits.

fruits eventually turn to a yellow color instead. The characterization of the carotenoids in this tomato mutant has not been reported previously.

Various tomato mutants have been used in the establishment of part of the biosynthetic pathways of carotenoids in higher plants (Mackinney and Jenkins, 1949; Porter and Lincoln, 1950). The desaturation of phytoene to lycopene series was the direct result of this type of study. Some doubts still exist to the point of cyclization from acyclic carotenes to cyclic carotenes in fruits. A recent review seems to favor the suggestion that cyclization at both the neurosporene and lycopene levels can take place (Goodwin, 1971). Two separate biosynthetic pathways for β-carotene and lycopene have been proposed on the basis of temperature inhibition (Tomes, 1963) and chemical inhibition (Raymundo *et al.*, 1967) studies in normal red tomatoes. Khudairi (1972) reviewed the ripening of tomatoes and presented a genetic interpretation of the carotene synthesis from various tomato mutants.

In this study, the carotenoids of the yellow and red lutescent tomatoes at several stages of ripeness are reported.

EXPERIMENTAL SECTION

Yellow and red lutescent tomatoes were grown in the greenhouse and harvested at the various stages of ripeness based on the ground color of the fruits. The carotenoids were isolated and the extracts were saponified as described by Davies (1965). Fruits weighing 150–200 g were used in each extraction. The extracts in petroleum ether (bp 30–60°) were dried over anhydrous sodium sulfate before being concentrated to small volume on a flash evaporator under reduced pressure and placed onto columns of magnesium oxide–hyflo super cell (1:2, w/w). The columns were premoistened with petroleum ether and developed with petroleum ether and 1–30% acetone in petroleum ether under suction. A large test tube was placed inside the suction flask to collect the eluates. A new tube was replaced whenever a band was eluted from the column. The eluted fractions were evaporated to dryness and redissolved in appropriate solvents for spectral measurements on a Perkin-Elmer Model 402 spectrophotometer. The individual carotenoids were identified by their position on the column and their visible spectra. The quantity of each carotenoid was calculated from published extinction coefficients (Davies, 1965). Fractions containing more than one carotenoid as judged by the spectra were rechromatographed, usually on alumina, to obtain further purification. Thin-layer chromatography was employed in identifying the following carotenoids with authentic carotenoids isolated from known sources: β-carotene (from carrot), neurosporene (from *Neurospora*), lycopene (from tomato), neoxanthin and lutein (from spinach). Plates used were precoated silica gel II (Eastman Kodak Chromagram No. 13179) developed with petroleum ether (bp 60–110°)–ethyl acetate–diethylamine (79:15:6, v/v). The solvent system was modified from Bolliger and Konig (1969) to analyze all carotenoids mentioned above on a single plate. All solvents used were of reagent grade except petroleum ether which was purified by passing through a silica gel column (Davison Grade H) until no absorption occurred beyond 230 nm against water blanks. Use of unpurified petroleum ether would result in the loss of the phytoene fraction on column and unsatisfactory thin-layer chromatographic separation. Spectra of pigments were obtained in spectrophotometric grade solvents.

RESULTS AND DISCUSSIONS

The yellow lutescent tomato fruits contained low amounts of carotenoids even at the ripe stage (Table I). The presence of phytoene was not detected at all. A trace of blue fluorescence compound, possibly phytofluene, was observed but was not able to be quantitized in ripe fruits. The major carotenes detected in yellow lutescent tomatoes were neurosporene and β-carotene which combined to account for about three-fourths of the total carotenoids. Only traces of lycopene was detected in ripe fruits. The isolated neurosporene fraction had the same visible spectrum as the neurosporene from *Neurospora* and they cochromatogramed on thin-layer plates. The R_f values of the carotenoids analyzed were: β-carotene, 0.90; neurosporene, 0.85; lycopene, 0.78; neoxanthin, 0.09; and lutein, 0.14. These results reflected some differences in the carotenoids between the yellow lutescent tomatoes and the normal yellow tomatoes (Mackinney and Jenkins, 1949; Zscheile, 1966). Neurosporene and xanthophylls were not reported in the normal yellow tomatoes.

The carotenoids of the red lutescent tomatoes were very close to those reported earlier by Raymundo *et al.* (1970). Detached mature white tomatoes were used in their study and the tomatoes were ripened under controlled conditions. β-Carotene was synthesized first but seemed to reach a plateau while lycopene accumulated rapidly when the tomatoes started to turn orange in color. When comparing the data reported by Raymundo *et al.* (1970) and Villegas *et al.* (1972) with the results in Table II it is seen that less β-carotene was observed in this study while more phytoene and phytofluene were observed. This could be the discrepancy between horticultural practices and ripening conditions. The existence of a small amount of neurosporene was detected but was in minute amount in comparison with other carotenes. Tomes (1963) and Raymundo *et al.* (1967) did not observe any neurosporene in normal red tomatoes. Besides neurosporene, all carotenes in the Porter and Lincoln scheme were observed and their quantities increased in rough proportions with advanced ripeness. Only small amounts of xanthophylls appeared to exist in red lutescent tomatoes and these were not identified.

The lutescent gene of the tomatoes seemed to provide only minor alterations of the composition of carotenoids compared to other genotypes (Khudairi, 1972). A conclusive statement could not be made at this time until more

crossing of the lutescent tomatoes to other genotypes was made. The fact that no chlorophyll was present at mature stages of these lutescent tomatoes provides an advantage for carotenoid study particularly in the biosynthetic pathways of carotenoids.

ACKNOWLEDGMENT

The author thanks G. Mackinney for seeds of the lutescent tomatoes, a gift of neurosporene sample, and comments on the manuscript. R. L. Thomas provided able technical assistance.

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Joseph J. Jen

Department of Food Science
Clemson University
Clemson, South Carolina 29631

Received for review April 20, 1973. Accepted April 1, 1974. Technical Contribution No. 1091 from the South Carolina Agricultural Experiment Station, Clemson, S. C. 29631.

In Vitro Metabolism of [^{14}C]Photodieldrin by Microsomal Mixed-Function Oxidase of Mouse, Rat, and House Flies

Metabolism of [^{14}C]photodieldrin, *in vitro*, was studied with microsomal mixed-function oxidase of rat, mice, and house fly. The results of these experiments indicate that photodieldrin is metabolized at very low levels in these animals. Qualitative analyses of metabolites showed variation with species and sex of the animal. There were three metabolic products in male and female mice but only two metabolites in male rat and none in the female. However, in female house flies only one metabolite was observed. The microsomal mixed-function oxidase inhibitor piperonyl butoxide blocked the formation of these photodieldrin metabolites.

The chlorinated cyclodiene insecticides, aldrin and dieldrin, are next to DDT in their usage and persistence in the environment. Their residues are commonly present in water, soil, plants, and in terrestrial and aquatic animals (Edwards, 1970). The residues of aldrin and dieldrin in soil, water, or on plant surfaces can be converted to photoaldrin and photodieldrin in the presence of sunlight, as well as by microorganisms (Robinson *et al.*, 1966; Rosen *et al.*, 1966; Harrison *et al.*, 1967; Lichtenstein *et al.*, 1970; Matsumura *et al.*, 1970; Ivie and Casida, 1971a,b; Klein *et al.*, 1973; Kohli *et al.*, 1973). Both aldrin and photoaldrin can be converted to photodieldrin by living organisms. Thus, aldrin and dieldrin can form the "terminal residue" of photodieldrin (Eagan, 1969) which is more toxic to aquatic and terrestrial animals (Rosen *et al.*, 1966; Rosen and Sutherland, 1967; FAO, 1968; Sutherland and Rosen, 1968; Khan *et al.*, 1970, 1973). The effects of this "terminal residue" as well as its fate in living organisms need to be investigated.

Although considerable attention has been paid to the studies of the metabolism of dieldrin in various animals (Matthews and Matsumura, 1969; Matthews *et al.*, 1971; McKinney *et al.*, 1972; Nelson and Matsumura, 1973) only a few reports are available on the metabolism of photodieldrin (Khan *et al.*, 1969; Klein *et al.*, 1970; Dailey *et al.*, 1972). Recently, Klein *et al.* (1970) showed the *in vivo* formation of ketodieldrin, "Klein's metabolite," in the male rat and of four unidentified polar metabolites in the female rat urine. *In vivo* metabolism of photodieldrin can be inhibited by an antioxidant synergist, sesamex (Khan *et al.*, 1970). Since sesamex in house flies is a known inhibitor of the microsomal mixed-function oxidase (MFO) the possibility of the *in vitro* metabolism of photodieldrin by this system exists. However, *in vitro* metabolism of photodieldrin has not been reported in animals. Therefore, we conducted the present experiments *in vitro* to study the metabolism of photodieldrin by MFO to understand the role of this system in the degradation of photodieldrin. The animals used in the present study include rats, mice, and house flies.

MATERIALS AND METHODS

[^{14}C]Photodieldrin was prepared by ultraviolet irradiation of [^{14}C]dieldrin (Mallinckrodt Chemicals and Amersham-Searle, sp act. 30 mCi/mmol). The final purified product (sp act. 4 μCi/μmol) was essentially free of interfering compounds as tested by electron-capture gas-liquid chromatography (glc) and thin-layer chromatography (tlc) followed by X-ray autoradiography. Nonradioactive photodieldrin was similarly prepared by ultraviolet irradiation of dieldrin (Shell Chemical Co., 99.5% pure) as described by Rosen and Carey (1968). Glucose 6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PD), and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from Sigma Chemical Co.

Rats (Charles River Breeding Laboratories) and mice (Scientific Small Animals) of both sexes of 8-9 weeks of age were fed on rat chow and water *ad libitum* and maintained at 12-15 hr of daylight. Female house flies of a resistant strain (*YFc*), 5-6 days old, were used in all experiments. They were fed on sugar, milk, and water *ad libitum*.