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.

LITERATURE CITED

Bollinger, H. R., Konig, A., "Thin layer Chromatography," Stahl, E., Ed., Springer-Verlag New York, New York, N. Y., 1969, p 266.
Bolliger, H. R., Konig, A., "Thin Layer Chromatography," Stahl, E., Ed., Springer-Verlag New York, New York, N. Y., 1969, p 266.
Davies, B. H., "Chemistry and Biochemistry of Plant Pigments," Goodwin, T. W., Ed., Academic Press, New York, N. Y., 1965, p 496.
Goodwin, T. W., "Carotenoids," Isler, O., Ed., Birkhauser Verlag, Basel, 1971, p 577.
Khudairi, A. K., *Amer. Sci.* **60**, 696 (1972).
Mackinney, G., *Qual. Plant. Mater. Veg.* **25**, 281 (1958).
Mackinney, G., Jenkins, J. A., *Proc. Nat. Acad. Sci. U. S.* **35**, 284 (1949).
Porter, J. W. Lincoln, R. E., *Arch. Biochem.* **27**, 390 (1950).
Raymundo, L. C., Griffiths, A. E., Simpson, K. L., *Phytochemistry* **6**, 1527 (1967).
Raymundo, L. C., Griffiths, A. E., Simpson, K. L., *Phytochemistry* **9**, 1239 (1970).
Tomes, J. L., *Bot. Gaz. (Chicago)* **124**, 180 (1963).
Villegas, C. N., Chichester, C. O., Raymundo, L. C., Simpson, K. L., *Plant Physiol.* **50**, 694 (1972).
Zscheile, F. P., Jr., *Arch. Biochem. Biophys.* **113**, 554 (1966).

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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*.

Table I. Radioactivity Recovered in Organic and Aqueous Phases after the Incubation of [^{14}C]Photodieldrin with the Microsomal Mixed-Function Oxidase

Animals	Experiment	mg of protein/ incubation	dpm added/ incubation	Organic phase[a] dpm recovered	Organic phase[a] % recovery	Aqueous phase[b] dpm recovered	Aqueous phase[b] % recovery
Male mouse	Control	0.35	41,000	31,816	78	58	0.14
	Sample	0.35	41,000	30,733	75	50	0.12
Female mouse	Control	0.72	41,000	38,400	93	468	1.10
	Sample	0.72	41,000	39,225	94	412	0.97
Male rat	Control	0.25	85,000	67,737	79	9	0.01
	Sample	0.25	85,000	64,466	75	9	0.01
Female rat	Control	0.45	85,000	68,000	80	680	0.80
	Sample	0.45	85,000	69,700	82	850	1.00
Female house fly	Control	0.42	85,000	66,583	78	255	0.30
	Sample	0.42	85,000	69,616	81	85	0.10

[a] Average values of three–four replicates. [b] The aqueous phases of three–four replicates were pooled, lyophilized, and then analyzed.

Mice or rats were killed by a blow on the head. Livers were taken out immediately and transformed to cold phosphate buffer (0.1 *M*, pH 7.4) and used for microsome preparation. Abdomens of female house flies were used for enzyme source. The microsomal preparation and enzyme assay were as described by Stanton and Khan (1973) and Khan *et al.* (1970).

Enzyme incubations were carried out aerobically in 25-ml erlenmeyer flasks at 37° for 2 hr in a shaker water bath. The incubation mixture (final volume of 3.3 ml) consisted of 0.6 m*M* $NADP^+$, 5.0 m*M* G-6-P, 2 units of G-6-PD, and about 200–800 μg of microsomal protein and substrate added in 100 μl of ethylene glycol monomethyl ether. The reaction was terminated by shaking with 6 ml of hexanes. The samples were then frozen for 24–36 hr, before extraction. Control experiments were carried out similarly but with boiled microsomes. All experiments were repeated at least two times using three–four replicates. Protein was estimated by the method of Lowry *et al.* (1951).

The incubation mixture was extracted two times with 6 ml of hexane followed by two times with 6 ml of diethyl ether. The ether and hexane extracts were pooled and evaporated in a gentle current of air. The residue was redissolved in 1 ml of hexane, which was used for liquid scintillation, thin-layer chromatography, and gas–liquid chromatography analyses. The organic phase of each individual replicate was analyzed on a liquid scintillation spectrometer (Model 3390 with a Model 544 absolute activity analyzer, Packard Instruments, Chicago, Ill.). The counting efficiency of the instrument with the Model 544 absolute activity analyzer was 99.8%. Preliminary experiments with each replicate when spotted on tlc plates showed a low level of detectable metabolites. Therefore, three–four replicates were pooled, concentrated, and then spotted on thin-layer plates. The aqueous phase of different replicates (three–four control or three–four experimental replicates) were pooled and lyophilized. The residue was transferred with ether and acetone washings. The solvent was evaporated to dryness and the residue redissolved in a known volume of acetone and then analyzed by liquid scintillation, glc, tlc, and autoradiography.

Tlc was accomplished on 0.25 mm thick silica gel F-254 precoated plates (E. Merck of Darmstadt, Germany). Samples, organic or aqueous phases, were spotted on plates and developed in benzene–ethyl acetate (3:1) which was routinely employed. After developing, the plates were exposed to X-ray No-Screen film (Kodak Eastman Co.) for 25–30 days. After developing the film, the darkened areas were noted and R_f values recorded. For radioactivity counts, samples (20–100 μl) were added to 10 ml of scintillation cocktail (Klein *et al.*, 1970).

A Packard gas chromatograph, Model 7300 series (outfitted with a 2 × $^1/_8$ in. glass column and packed with 6% DC-200 on Varoport-30), equipped with a Ni-63 electron capture detector was employed for glc analysis. The following conditions were employed: inlet temperature, 220°; column, 210°; detector, 220°; N_2 flow rate, 25 ml/min; sensitivity, 1 × 10^{-10}.

RESULTS AND DISCUSSION

Initial *in vitro* experiments showed that about 8% of the nonradioactive photodieldrin is metabolized by mouse hepatic mixed-function oxidase. Incubation of 90 μg of "cold" photodieldrin resulted in the recovery of 72 μg as compared to 80 μg in the control as analyzed by glc. The metabolic products formed could not be detected by this method. However, the analysis of the organic phase of the incubation carried out with [^{14}C]photodieldrin showed no difference in the recovered radioactivity between control and experimental replicates (Table I) suggesting the metabolite(s) were soluble in the organic phase. Only about 1% of radioactivity was detected in the aqueous phase of control and experimental samples suggesting the presence of very low levels of unextractable photodieldrin or its hydrophilic metabolites which could have been produced nonenzymically.

The organic phases from mouse, rat, and house flies, when analyzed by tlc followed by autoradiography, showed the presence of metabolic products other than photodieldrin (Figure 1). It can be seen that there are a

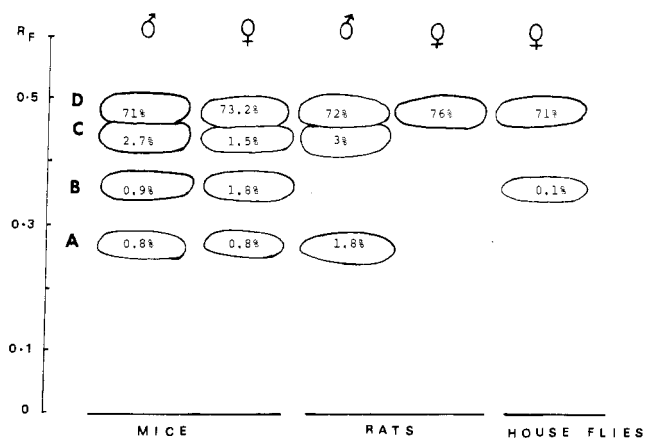

Figure 1. Thin-layer chromatographic presentation of [^{14}C]photodieldrin and its *in vitro* metabolites by various animals. Spots A, B, and C are unidentified metabolites. Spot D represents photodieldrin. No such metabolic products were seen in the control (not shown in the figure). Organic phase of three–four replicates spotted on tlc plates and developed in solvent system benzene—ethyl acetate (3:1) and exposed to X-ray film for 25–30 days.

number of metabolites: three in the mouse, two in the rat, and one in the house fly. They are designated A, B, C (metabolites), and D (photodieldrin). The R_f values of A, B, C, and D are 0.27, 0.36, 0.43, and 0.48, respectively, in benzene–ethyl acetate (3:1) solvent system. The metabolites on tlc plates, corresponding to the darkened areas of the X-ray film, were scraped off and extracted with acetone for liquid scintillation counting and glc analysis. The per cent recoveries of photodieldrin or its metabolites are given in Figure 1. Our attempts to detect these extracted metabolites by glc using various columns (6% DC-200 on Varaport-30, 5% QF-1 and 3% SE-30 or SE-52 on Chromosorb W) were not successful. The reason for nondetectability on glc may be due to either low levels or nonvolatile nature or nonelution of the compounds under these conditions. Similar observations have been reported in the case of [^{14}C]photodieldrin metabolites *in vivo* (Klein *et al.*, 1970).

That these metabolites are enzymatic products of the mixed-function oxidase was confirmed by the lack of metabolism in the absence of NADP or inhibition of the metabolism in the presence of 10 μg of piperonyl butoxide (an inhibitor of MFO) in the incubation.

There were no qualitative differences in the metabolic products formed between male and female mice. However, only the male and not the female rat showed the degradation of photodieldrin. This may be related with higher levels of MFO activity in the male rat. For example, the specific activities (picomoles/microgram of protein per minute) of epoxidation of aldrin and photoaldrin were, respectively, 3.6 and 4.2 by male and 0.5 and 0.8 by female rat MFO. Such differences in MFO activity toward cyclodienes between male and female rats have been reported by other workers (Wong and Terriere, 1965). Differences between male and female rats in the *in vivo* metabolism, distribution, and excretion of photodieldrin have also been observed by several workers (Klein *et al.*, 1970; Dailey *et al.*, 1970, 1972).

The present investigation thus provides evidence that photodieldrin is oxidatively metabolized to lipophilic products by rat, mouse, and house fly mixed-function oxidase. The chemical nature of these metabolites and their toxicity should be investigated to understand the significance of this metabolic conversion.

LITERATURE CITED

Dailey, R. E., Klein, A. K., Brouwer, E., Link, J. D., Braunberg, R. C., *J. Agr. Food Chem.* **20**, 371 (1972).
Dailey, R. E., Walton, M. S., Beck, V., Leavens, C. L., Klein, A. K., *J. Agr. Food Chem.* **18**, 433 (1970).
Eagan, H., *J. Ass. Offic. Anal. Chem.* **52**, 299 (1969).
Edwards, C. A., "Persistent Pesticides in the Environment," Chemical Rubber Publishing Co., Cleveland, Ohio, 1970.
Food and Agriculture Organization of The United Nations World Health Organization, "1967 Evaluations of Some Pesticide Residues in Food," FAO/PI, 1967/M/11/1, WHO/Food Additives 68-30, Rome, 1968, p 104.
Harrison, R. B., Holmes, D. C., Roburn, J., Tatton, J. O. G., *J. Sci. Food. Agr.* **18**, 10 (1967).
Ivie, G. W., Casida, J. E., *J. Agr. Food Chem.* **19**, 405 (1971a).
Ivie, G. W., Casida, J. E., *J. Agr. Food Chem.* **19**, 410 (1971b).
Khan, M. A. Q., Rosen, J. D., Sutherland, D. J., *Science* **168**, 318 (1969).
Khan, M. A. Q., Stanton, R. H., Sutherland, D. J., Rosen, J. D., Maitra, N., *Arch. Environ. Contam. Toxicol.* **1**, 159 (1973).
Khan, M. A. Q., Sutherland, D. J., Rosen, J. D., Carey, W. F., *J. Econ. Entomol.* **63**, 470 (1970).
Klein, A. K., Dailey, R. E., Walton, M. S., Beck, V., Link, J. D., *J. Agr. Food Chem.* **18**, 705 (1970).
Klein, W., Kohli, J., Weisgerber, I., Korte, F., *J. Agr. Food Chem.* **21**, 152 (1973).
Kohli, J., Zarif, S., Weisgerber, I., Klein, W., Korte, F., *J. Agr. Food Chem.* **21**, 855 (1973).
Lichtenstein, E. P., Schulz, K. R., Fuhremann, T. W., Liang, T. T., *J. Agr. Food Chem.* **18**, 100 (1970).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, K., *J. Biol. Chem.* **193**, 265 (1951).
Matsumura, F., Patil, K. C., Boush, G. M., *Science* **170**, 1208 (1970).
Matthews, H. B., Matsumura, F., *J. Agr. Food Chem.* **17**, 845 (1969).
Matthews, H. B., McKinney, J. D., Lucier, G. W., *J. Agr. Food Chem.* **19**, 1244 (1971).
McKinney, J. D., Matthews, H. B., Fishbein, L., *J. Agr. Food Chem.* **20**, 597 (1972).
Nelson, J. O., Matsumura, F., *Arch. Environ. Contam. Toxicol.* **1**, 224 (1973).
Robinson, J., Richardson, A., Bush, B., *Bull. Environ. Contam. Toxicol.* **1**, 127 (1966).
Rosen, J. D., Carey, W. F., *J. Agr. Food Chem.* **16**, 536 (1968).
Rosen, J. D., Sutherland, D. J., *Bull. Environ. Contam. Toxicol.* **2**, 1 (1967).
Rosen, J. D., Sutherland, D. J., Lipton, G. R., *Bull. Environ. Contam. Toxicol.* **1**, 133 (1966).
Stanton, R. H., Khan, M. A. Q., *Pestic. Biochem. Physiol.* **3**, 351 (1973).
Sutherland, D. J., Rosen, D. J., *Mosquito News* **28**, 1955 (1968).
Wong, D. T., Terriere, L. C., *Biochem. Pharmacol.* **14**, 375 (1965).

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Received for review December 31, 1973. Accepted May 11, 1974. This study was supported by Grant ES00808 from the National Institute of Environmental Health Sciences.

Alkylthiazoles in Potato Products

2,4,5-Trimethylthiazole and 2-isopropyl-4,5-dimethylthiazole have been characterized in the basic fraction of the volatile oil of potato chips using the combination of capillary gas chromatography and mass spectrometry. The above compounds and the additional compounds 2-isopropyl-4-methyl-5-ethylthiazole, 2-isobutyl-4,5-dimethylthiazole, and 2-acetylthiazole have also been characterized in the basic fraction of the volatile oil from boiled potatoes.

A number of alkyl- and alkanoylthiazoles have been found in foods, particularly roasted foods. Those found up to the present are summarized in Table I. Other related compounds that have been found in foods are 2-acetylthiazoline (Tonsbeek *et al.*, 1971) in beef broth and benzothiazole in a great variety of foods.

In the authors' studies of the volatile components of potato products over the last several years a number of alkylthiazoles have been detected. Because of the potent and characteristic odor of some alkylthiazoles these compounds could be important to the aroma and flavor of potato products.