Effects of Pesticides on Active Transport of Glucose through the Isolated Intestine of the Mouse

The effects of a number of pesticides were examined on active transport of glucose through the isolated intestine of the mouse. Inhibition of active transport was apparent for all of the fungicides tested. A majority of the herbicides were also inhibitory, and slightly less than one-half of the insecticides gave this type of action. Although an inhibitory effect was usually supported by previously reported inhibition of mitochondrial activity, a number of compounds which are known to uncouple oxidative phosphorylation or interrupt electron transport did not affect active transport of glucose in these experiments.

Active transport of glucose by the mammalian intestine was first shown by Czaky (1942–1943) and has been confirmed by others (Wilson and Vincent, 1965; Crane, 1965). It is accepted generally (Cirillo, 1972) that active sugar transport by animal tissues may be inhibited at three levels: (a) inhibition of sugar carrier function (phlorizin), (b) inhibition of the Na^+–K^+-activated ATPase (ouabain), or (c) inhibition of ATP synthesis (DNP). Considerable care must be exercised in interpreting data obtained with inhibitors because many are not very specific in their action, and the type of action expressed is often related to concentration. Active transport requires an input of energy supplied by mitochondrial oxidative phosphorylation. Compounds such as amytal, rotenone, actimycin A, cyanide, and oligomycin, which like DNP interfere with oxidative phosphorylation (Slater, 1967) of isolated mitochondria, also can be expected to inhibit active transport by limiting the availability of energy, if they should penetrate into the tissue and reach the mitochondria.

A number of pesticides are known to affect oxidative phosphorylation of isolated mitochondria (Moreland, 1967; Casida, 1972; Owens, 1963). However, the effects of these compounds on transport of glucose in the mammalian midgut have not been investigated. It seemed appropriate, therefore, to examine a number of pesticides known to interfere with respiration by evaluating their action on active transport of glucose in preliminary experiments.

MATERIALS AND METHODS

The pesticides utilized had a purity of 95% or more (except technical toxaphene and chlordane) and included: DDT, dieldrin, toxaphene, chlordane, mirex, endrin, endosulfan, dimethoate, diazinon, azinphosmethyl, parathion, malathion, carbaryl, rotenone, piperonyl butoxide, sodium azide, ioxynil, oryzalin, C-9122 [3,5-dibromo-4-hydroxybenzaldehyde-*O*-(2,4-dinitrophenyl)oxime], HOQNO (2-heptyl-4-hydroxyquinoline *N*-oxide), 2,4,5-T, diuron, atrazine, propanil, chlorpropham, DNP, dithane, chlorthalonil, chlorneb, benlate, and triphenyltin hydroxide.

The procedures utilized for the midgut preparations have been described elsewhere (Shah and Guthrie, 1970). Briefly, a 4-cm section of the small intestine of 6-week-old male mice starved for 24 hr was removed and placed in pH 7.0 phosphate buffer (minus glucose). The aboral end was ligated with cotton thread and the preparation placed in 30 ml of buffer (serosal fluid) in a 50-ml centrifuge tube with connections at the oral end of the gut for introduction of solutions into the mucosal side and for aeration. Aliquots (0.25 ml) of solutions or suspensions (0.1 m*M*) of the unlabeled pesticides prepared in buffer modified by addition of 0.3% acetone and 0.03% Tween 80 were introduced simultaneously with 0.9 n*M* D-[U-^{14}C]glucose. The amount of radioactivity found in the serosal and luminal fluids, and that associated with the gut tissue, was determined after incubation for 80 min at 30°. Each experiment was repeated four times.

An analysis of variance was performed using the pooled variation among replications as an estimate of experimental error. The least significant difference (LSD) for comparing chemicals with the glucose control was computed from the error variance for two levels of significance (0.05 and 0.01).

RESULTS AND DISCUSSION

Effects of pesticides on the penetration of [^{14}C]glucose across the isolated intestine of the mouse are shown in Table I. DNP, which was included as a classical uncoupler of oxidative phosphorylation, inhibited transport by 39%. HOQNO and sodium azide, which inhibit mitochondrial electron transport by acting at complexes III and IV, respectively, were included to determine the action of other classical inhibitors of mitochondrial reactions on glucose transport. Sodium azide was an even better inhibitor than DNP; however, HOQNO essentially had no effect. Rotenone, which was also used as a standard inhibitor of mitochondrial electron transport (acts at complex I), also was not inhibitory to glucose transport.

Among the chlorinated hydrocarbon insecticides, neither mirex nor endrin significantly inhibited active transport, whereas dieldrin, toxaphene, chlordane, and endosulfan were equal to DNP in inhibitory action, and inhibition by DDT was slightly less than the other chlorinated compounds. It is interesting to note that the two chlorinated hydrocarbons that had the least effect on energy metabolism were also the least inhibitory of glucose transport. Among the phosphate insecticides, only dimethoate and azinphosmethyl showed significant inhibition. Members of other groups of insecticides (carbaryl, rotenone, and piperonyl butoxide) produced results that were not different significantly from the untreated control. All of the herbicides tested, except C-9122 and 2,4,5-T, were equal to DNP in their ability to inhibit glucose transport. All fungicides tested were significant in reducing transport of glucose.

These data suggest that glucose transport may be less affected by strong inhibitors of electron transport (sodium azide the only exception in the limited study reported herein) than by strong uncouplers of oxidative phosphorylation. Lack of interference of glucose transport by some compounds known to interfere with oxidative phosphorylation (such as C-9122) may be related to the failure of the compounds to penetrate the intestine. No direct measurement of pesticide concentration in the tissue was made in these studies. The lack of documentation of action of fungicides on mitochondrial activity makes correlation with energy metabolism difficult.

Gut association of glucose in transport did not correlate consistently with total penetration (into serosal fluid). Some compounds that inhibited active transport had no

Table I. Effects of Pesticides on Penetration of [^{14}C]Glucose across Isolated Intestine of Mouse (80 min)

Treatment	% radioact. for sample from: Serosal fluid	Gut associated	Luminal fluid	Effect on mitochondrial act.
Glucose	34.4	10.2	55.4	
Miscellaneous				
DNP	21.0**	11.4	69.4**	Uncouples O.P.[a] (Slater, 1967)
Sodium azide	16.6**	14.8*	68.6**	Inhibits E.T.[a] at complex IV (Slater, 1967)
HOQNO	38.1	9.6	52.4	Inhibits E.T. at complex III (Slater, 1967)
Insecticides				
DDT	26.4*	11.0	62.5*	Inhibits Mg^{2+} ATPase and E.T. (Cutkomp *et al.*, 1971; Waddill and Keeley, 1971)
Dieldrin	21.4**	12.9	65.7**	Inhibits cytochrome oxidase and succinic dehydrogenase (Nelson and Williams, 1971; Moffett and Yarbrough, 1972)
Toxaphene	23.0**	8.9	68.5**	Inhibits NADPH oxidase and succinooxidase (Pardini *et al.*, 1971)
Chlordane	25.6*	14.6*	58.5	Inhibits NADPH oxidase and succinooxidase (Pardini *et al.*, 1971)
Mirex	27.2	10.5	62.5*	Does not inhibit O.P. (Nelson and Williams, 1971)
Endrin	34.4	10.7	54.9	Marginally inhibits succinooxidase (Pardini *et al.*, 1971)
Endosulfán	22.7**	14.0	63.4*	No report
Dimethoate	25.1*	14.8*	63.0*	No report
Diazinon	27.4	9.4	63.2*	No report
Azinphosmethyl	24.4**	19.0**	56.7	No report
Parathion	27.4	11.8	60.7	No report
Malathion	35.0	8.7	57.3	No inhibition of Mg^{2+} ATPase (Cutkomp *et al.*, 1971)
Carbaryl	31.1	17.2**	52.5	No inhibition of Mg^{2+} ATPase (Cutkomp *et al.*, 1971)
Rotenone	30.8	12.1	57.2	Inhibits E.T. at complex I (Casida, 1972)
Piperonyl butoxide	27.4	11.4	61.7*	Marginally inhibits E.T. (Nelson *et al.*, 1971)
Herbicides				
Ioxynil	15.7**	19.0**	65.3**	Uncouples O.P. (Kerr and Wain, 1964; Ferrari and Moreland, 1969)
Oryzalin	24.5**	10.8	64.6**	Uncouples O.P. (Moreland *et al.*, 1972)
C-9122	28.3	9.9	65.4**	Uncouples O.P. (Moreland and Blackmon, 1970)
2,4,5-T	27.9	15.9**	55.9	Uncouples O.P. (Switzer, 1957)
Diuron	21.3**	18.1**	60.6	Uncouples O.P. (Foy and Penner, 1965)
Atrazine	26.4*	16.0**	57.6	Inhibits succinate oxidation (Foy and Penner, 1965)
Propanil	21.4**	9.4	69.2**	Uncouples O.P. (Hofstra and Switzer, 1968)
Chlorpropham	23.6**	14.1*	62.2*	Uncouples O.P. (Lotlikar *et al.*, 1968)
Fungicides				
Dithane	15.9**	13.2	70.4**	Inhibits phosphate dehydrogenases and citrate metabolism (Thorn and Ludwig, 1962; Owens, 1963)
Chlorthalonil	17.0**	16.0**	64.7**	No report
Chlorneb	26.1*	15.8**	61.7*	Respiration unaffected (Sisler, 1969)
Benlate	22.9**	16.3**	65.5**	Respiration unaffected (Sisler, 1969)
Triphenyltin	24.5**	14.8*	68.9**	Alkyltins uncouple O.P. (Aldridge and Street, 1970)
LSD 0.05*	7.3	3.9	6.3	
0.01**	9.6	5.2	8.3	

[a] The abbreviations O.P. for oxidative phosphorylation and E.T. for electron transport are used throughout this table.

effect on the glucose associated with the gut tissue (DDT, oryzalin, and dithane for example), whereas other compounds which inhibited active transport showed an increase in the glucose associated with the gut tissues (chlordane, azinphosmethyl, ioxynil, and benlate for example). However, some compounds which did not inhibit active transport also did not affect gut association (mirex, endrin, diazinon, parathion, malathion, rotenone, piperonyl butoxide, C-9122, HOQNO), but others significantly affected gut association (carbaryl and 2,4,5-T). Increased quantities of radioactivity in the lumen were usually related to decreased serosal transport, although there were some exceptions. Correlation among these effects is complicated by both high initial concentration and diffusion back into the lumen.

Lack of inhibition of glucose transport did not correlate consistently with water or lipid solubility. For example, some pesticides with limited solubility in water did not inhibit, whereas others with essentially no solubility did inhibit.

Inhibition of the transport process may result either from inhibition of the specific sugar-protein reaction or by modification of the membrane structure. Pesticides which inhibit oxidative phosphorylation usually inhibited glucose transport, but there were exceptions. Conclusions are less evident for events at the electron transport level and on ATPases *per se*. It is possible that interactions of pesticides with carrier proteins could change the configuration of the macromolecule and alter glucose transport in a manner unrelated to metabolism. Also, the more fat soluble pesticides could modify the membrane structures and affect transport in a different, unspecific manner. It seems obvious that no single mechanism can be identified presently for alteration of glucose transport by pesticides. Although the action of pesticides on glucose transport would contribute in a minor way to the chronic toxicity of pesticides that are metabolized rapidly, pesticides which tend to be stored could exert a long term effect on glucose transport thereby contributing to a chronic action.

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Major Carotenoids of the Seeds of Three Cultivars of the Tomato, *Lycopersicon esculentum* L.

Carotenoids from the seeds of three tomato cultivars were separated by chromatography and identified by absorption spectroscopy. Lutein, β-carotene, and lycopene were the major pigments of Chico Grande and Rutgers seeds, and lutein, β-carotene, and ζ-carotene were the major pigments of Golden Jubilee seeds. Xanthophyll pigments predominated over carotenes in the seeds of all cultivars. β-Carotene was predominate over lycopene in the seeds of both red fruited cultivars and over ζ-carotene in the seeds of the yellow fruited cultivar.

The carotenoid composition of tomato fruit and the inheritance of fruit color of many species and cultivars of tomatoes have been studied extensively since the early 1930's (Kuhn and Grundmann, 1932). However, the pigment composition of tomato seeds has not been reported. It was the purpose of this study to identify and quantify the seed pigments from seeds of three different tomato cultivars and to compare the results with known pigments of the fruit.

MATERIALS AND METHODS

Dried seeds (4.5 kg) of each of the three cultivars (Chico Grande, Rutgers, and Golden Jubilee) were prepared for pigment analysis. Seeds were cleaned to remove all visible foreign matter and fragments of skin, and the whole seeds were washed three times with methylene chloride solvent. Following solvent washing, seeds were ground and oils were extracted with methylene chloride. After removal of solvents, residues were saponified and partitioned between petroleum ether and 95% methanol (Goodwin, 1965). Epiphase and hypohase pigments were chromatographed separately using increasing amounts of acetone in petroleum ether ranging from 0 to 35% acetone. Epiphase pigments were chromatographed on a column of alumina (Baker) and hypophase pigments were separated on a column of equal parts of magnesium oxide (Baker) and Hyflo-Super-Cel (Johns Manville). The alumina column separations were by gravity flow, while 5–10 psi air pressure was used with the magnesium oxide columns.

Individual pigments were characterized by their absorption spectra in the range of 180–550 nm using both hexane and petroleum ether, by comparison of absorption maxima with literature values (Goodwin, 1965), and by comparison of their spectra with those of pure crystalline carotenoids. Crystalline lycopene and lutein (3,3′-dihydroxy-α-carotene) were isolated from tomato paste and spinach leaves, respectively, synthetic *all-trans*-β-carotene was obtained from Hoffman-La Roche, and the ζ-carotene (7,8,7′,8′-tetrahydrolycopene) spectrum was compared to the spectrum published by Nash and Zscheile (1945). Spectral data were obtained using 1.0-cm matched cells with a Coleman-Perkin-Elmer Model 124 ratio recording spectrophotometer and Model 165 recorder. Quantitative estimations were made in the usual manner using absorbance (Gillam and Stern, 1958).

RESULTS AND DISCUSSION

Solvent washing of the whole tomato seeds was necessary to ensure that subsequent analyses for seed pigments did not include pigments of the fruit that might have adhered to the outside of the seeds. However, when washing residues were examined spectrophotometrically, no materials absorbing visible light were found. There was a considerable amount of waxy material removed from the seeds by solvent washing. Subsequent saponification of this material showed a uniform unsaponifiable fraction among the three cultivars (Table I). The unsaponifiable fractions of the oils were more variable in quantity, rang-