THE TRANSFER OF ENDRIN VIA THE MILK TO PINE MOUSE PUPS AND THE RESULTANT EFFECT ON HEPATIC MICROSOMAL ACTIVITY

by

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MFO	mixed function oxidase
GLC	gas liquid chromatograph
F	fahrenheit
DMSO	dimethyl sulphoxide
S.E.M.	standard error of the mean
P	probability

INTRODUCTION

Pesticides have been used on a world wide basis over the past twenty years. Due to repeated selection through the man made pressure of pesticide use, certain target species now have populations which exhibit a certain degree of resistance to a particular pesticide. Examples of mammalian resistance to pesticides are rare in the literature. Boyle has shown that the rat species Rattus norvegicus is somewhat resistant to warfarin, an anticoagulant (1). Ozburn and Morrison reported a slight tolerance to DDT by the laboratory mouse, Mus musculus, after imposition of DDT pressure to several generations in the laboratory (2). In a very similar experiment, Gutherie and co-workers were unable to produce the tolerance seen by Ozburn and Morrison (3).

Endrin, a chlorinated hydrocarbon, has been used to control the species Microtus pitymus pinetorum, commonly called pine mice, which is an apple orchard pest. Webb and Horsfall have shown the existance of endrin resistant populations of pine mice (4). These animals were captured from orchards where endrin had been used as a controlling agent for ten years and exhibited a twelve fold higher LD_{50} value for endrin when compared to the LD_{50} value for pine mice live trapped from orchards where endrin was never used. Further toxicity studies showed a continued resistant state for progeny of resistant animals raised in pesticide free conditions (5).

The biochemical mechanism for resistance has been under investigation in the laboratory using progeny of live trapped endrin

resistant and susceptible mice. Resistant breeding pairs have continuously been selected for by breeding survivors of a 20 mg/kg oral dose of endrin. This is done to insure the maintenance of the resistant strain under laboratory conditions. The progeny of both strains are used in experiments so as to have control over the conditions the animals face and the age of the animals used.

The purpose of the investigation to be presented in this paper is to further clarify whether the resistance phenomenon is an inherited trait or an acquired trait which must be renewed with each generation by endrin stress. Since F_1 progeny exhibit the resistance phenomenom the question that is asked is whether this is due to a genetic trait passed to the offspring or whether enough residual endrin is present in the milk of the resistant dam to create an acquired resistance in the pup.

LITERATURE REVIEW

Hepatic MFO Activity - The hepatic mixed function oxidase (MFO) system is the major drug metabolizing enzyme system in mammalian organisms. Several review articles (6,7,8,9,10,11) have been written concerning the action of the hepatic MFO system in the detoxification of numerous drugs. Implicit in these papers is the fact that the MFO system is very nonspecific. Various drugs and other foreign compounds which are metabolized by the hepatic MFO system have the capacity to induce MFO activity. Compounds such as phenobarbital are broad spectrum inducers causing increased metabolism of a wide range of foreign compounds. Benzpyrene, on the other hand, causes increased metabolism of a narrow group of foreign compounds. In either case pretreatment of an animal with sub-lethal amounts of an inducing agent may serve to give the animal increased tolerance to that specific agent and possibly to other toxic agents.

Certain pesticides are included in those groups of toxic agents which can be metabolized to less toxic compounds by the hepatic MFO system. Therefore, the levels of hepatic MFO activity may determine whether or not an organism is able to survive exposure to a lethal dose of a pesticide. Because the levels of hepatic MFO activity can be induced by exposure to sub-lethal doses of a pesticide (12), an increased tolerance to the toxic effects of a pesticide may be developed.

MFO Activity of the Neonate - The hepatic MFO activity in the neonate is quite low in relation to the activity in mature animals.

Consequently the neonate does not have the capacity that the mature animal has to metabolize foreign compounds (13). Several studies have been done to determine the development of hepatic MFO activity in the neonate by using the post mitochondrial supernatant. Hart and Fouts (14) demonstrated that hepatic MFO activity for newborn rabbits was considerably lower than adult level activities. In studying the development of aminopyrine-N-demethylase activity in rats, Henderson (15) found a linear increase in activity for the first 20 days after birth followed by a more rapid increase, during which time the activity increased four fold, between days 20 and 40. Basu et al. (16) found that the development of nitrobenzoate reductase activity in rats closely paralleled the development of aminopyrine-N-demethylase activity as determined by Henderson (15). They also found, however, that biphenyl-4-hydroxylase and biphenyl-2-hydroxylase activities peaked at 25 days after birth with a very sharp increase coming between day 10 and day 25.

In subsequent studies microsomal activity was measured using a suspension of the microsomal pellet. Short and Stith (17) studied the development of ethylmorphine-N-demethylase activity and cytochrome P-450 content in newborn swine. Both the ethylmorphine-N-demethylase activity and the P-450 content showed a steady increase from one to six weeks after birth. Macleod and co-workers (18) found similar results with aminopyrine-N-demethylase activity in rats. Their results show a rapid increase in activity from one to five weeks of age with the activity peaking at ten weeks of age.

Cytochrome P-450 levels, however, reached a maximum three weeks after birth. Working with a different strain of rat, Uehleke and co-workers (19) obtained a parallel increase in aniline hydroxylase activity and cytochrome P-450 content with both levels plateauing 30 days after birth.

These studies all show a reduced hepatic MFO capacity for at least the first four weeks after birth. Compounds which are detoxified by the hepatic MFO system would be acted upon to a lesser extent in these animals than in mature animals. Consequently, the neonate would be more susceptible to the toxic effects of those pesticides which are detoxified by the hepatic MFO system.

Stimulation of MFO Activity in the Neonate - The hepatic MFO activity of adults can be stimulated by treating the animals with a variety of compounds such as barbiturates, pesticides and carcinogens (20). Research has been conducted to determine the effect such agents have on the hepatic MFO activity of the neonate.

In studies with pregnant rabbits, Hart and co-workers (14) treated with phenobarbital for three days prior to term and then determined the MFO activity of the newborn 24 hours after birth. A several fold increase over control values was found for aminopyrine demethylase and hexobarbital oxidase activity. In another study 12 day old rats which had been treated with phenobarbital developed a 161% increase in biphenyl-4-hydroxylase activity, a 260% increase in biphenyl-2-hydroxylase activity and an 84% increase in cytochrome P-450 content (16). It was also noted that

the per cent increase was higher for 12 day old rats as opposed to 21 and 52 day old rats. Darby (13) dosed lactating female rats with phenobarbitone and studied its effect on the MFO activity of the suckling pups. Ethylmorphine-N-demethylase activity increased three fold over control values, phenobarbitone hydroxylase activity increased five fold and aniline hydroxylase activity and cytochrome P-450 content increased 40%. This study indicates the ability of the neonate to react to an inducing agent present in the milk.

Although the hepatic MFO activity is low in the first few weeks after birth, the activity can be stimulated with known inducers of hepatic MFO activity. This stimulation can be accomplished by directly dosing the pup or indirectly by placental transfer and/or transfer via the milk. Indirect stimulation can only occur if sufficient amounts of the inducing agent are transferred from the female to the pup (13).

Pesticide Content in the Neonate - Certain pesticides are very persistant and therefore can eventually be consumed by mammals. DDT and other lipophilic pesticides can also be stored and accumulated in the fat of mammals. One means of excretion of these lipophilic pesticides would be through the milk of lactating females. In studies with human subjects, Kroger (21) has shown the presence of DDT, DDE, lindane and heptachlor epoxide in milk from these subjects. The amounts found were higher than the minimum allowed to be present in cow's milk.

In controlled laboratory studies with rats the presence of DDT and its metabolites was determined in newborn rats from dams maintained on a diet containing DDT (22). From this study it was found that newborn rats that had been allowed to suckle had five times the amount of DDT and its metabolites than did those rats sacrificed immediately after birth. This indicates that much more of the insecticide is transferred to the pup via the milk as opposed to placental transfer. In a similar study (23) milk from lactating female rats was analyzed for DDT and related products, after the rats had been maintained on a diet containing DDT. Greater amounts of DDT and its metabolites were found in milk tested on days one and three after birth of the pup as opposed to the amounts found on days six and ten.

It is evident that the suckling pup will consume certain levels of pesticides such as DDT, lindane, dieldrin and heptachlor epoxide due to their presence in the milk. If large enough amounts are present in the milk then possibly they could serve to alter the MFO activity in the suckling pup.

Pesticide Effects on MFO Activity - Although the effect of pesticides upon the hepatic MFO activity of the neonate has not been explored until recently (24), there is considerable data on the effects of pesticides on the hepatic MFO activity of adult animals. Chlordane, for instance, has been shown to be a broad spectrum inducer of hepatic MFO activity in an analogous manner to phenobarbital (12). DDT has been shown to increase hepatic MFO

activity in the rat (25). Recent evidence, however, has shown the effects of pesticide administration upon MFO activity to be a more complicated phenomenon.

Chhabra and Fouts (26) have found that mice are not as responsive to DDT stimulation as rats. In order to see pronounced increases of hepatic MFO activity with mice it was necessary to give high level i.p. doses of DDT in relation to LD_{50} values for the mice. Japanese Quail and mice also show a difference in response to DDT and DDE administration. DDE is a more potent stimulator of hepatic MFO activity in the Japanese Quail than is DDT (27) whereas the reverse is true for mice (28). Another DDT metabolite DDMU, causes an increase in ethylmorphine-N-demethylase activity in Japanese Quail which contrasts to a decrease in the demethylase activity when rats are treated with DDMU (27). Ducks and chickens also exhibit a difference in reaction to DDT administration. Aniline hydroxylase activity is induced in ducks receiving DDT treatment which contrasts to a decrease in the hydroxylase activity in chickens given the same DDT treatment (29). In this same study dieldrin was shown to have no effect on aniline hydroxylase activity in either species.

It seems plausible to think that the MFO activity will be altered in the neonate if enough of a particular pesticide is transferred via the milk. Recent work shows an induction in MFO activity in neonatal rats from dams treated with dieldrin (24). However in view of the work done with mature animals with various

pesticides, it would seem necessary to perform similar experiments with different species to avoid the mistake of incorrect extrapolation from one species to another.

MFO Activity in Pine Mice - Benzpyrene hydroxylase activity was used to determine the level of MFO activity in endrin resistant and susceptible pine mice. Resistant pine mice exhibited two fold higher activity than did the susceptible mice (30). Because these animals were from the orchard, there is the possibility that the difference in activities was due to residual endrin. Further work by Hartgrove and Webb (31) tends to diminish this possibility. In their studies with young progeny of orchard animals, they demonstrated that resistant pups had a two fold higher benzpyrene hydroxylase activity than did susceptible pups. Presumably these animals would not have come under endrin stress because of the pesticide free conditions in the laboratory in which they were raised.

There is, however, the possibility that residual endrin from the orchard will be present in the resistant dam. Passage of the residual endrin from the resistant dam to the pup may be sufficient to account for the two fold difference in the hydroxylase activity. The susceptible dams, on the other hand, were never exposed to endrin in the orchard and, consequently, there would be no endrin present in the milk which might cause an increase in MFO activity. This argument forms the initial basis for this study.

EXPERIMENTAL PROCEDURE

Materials

Animals - All mating pairs were progeny of orchard trapped animals. Resistant animals were trapped from an orchard near Berryville, Virginia, while susceptible animals were trapped near Sperryville, Virginia. The resistant progeny used as mating pairs were survivors of an oral dose of 20 mg of endrin per kg body weight.

The animals were maintained in stainless steel, wire bottomed cages and were fed Wayne Lab Blox purchased from Flow Laboratories in Dublin, Virginia. Washed burlap strips were added to the cages to serve as bedding. In all instances the animals were fed ad libitum. The laboratory was maintained at a constant temperature of 74° F with controlled lighting giving a 14 hour photoperiod.

Chemicals - Endrin (99+%) was obtained from Supelco, Belfonte, Pennsylvania. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and $NADP^+$ were purchased from Calbiochem, La Jolla, California. Tris buffer was obtained from Sigma Chemical Company, St. Louis, Missouri. Ethylmorphine hydrochloride was purchased from Merck and Company, Rahway, New Jersey. Aniline hydrochloride was purchased from Eastman Kodak, Rochester, New York. Celite 545, Florisil (60-100 mesh), and dimethyl sulphoxide (DMSO) spectranalyzed were purchased from Fisher Scientific Company, Fair Lawn, New Jersey. Three per cent OV-17 on 80/100 mesh Supelcoport and 1% OV-225 on 80/100 mesh Supelcoport

were purchased from Supelco, Belfonte, Pennsylvania. All other chemicals were reagent grade purchased from Fisher Scientific Company.

Methods

Endrin Transfer Studies - Endrin treatment to resistant and susceptible dams was initiated 24 hours after the birth of the offspring. In one study both resistant and susceptible dams received an oral dose of endrin in corn oil at a level of 0.51 mg/kg once daily for three consecutive days. Pups from these dams were sacrificed 24 hours after the last dose was administered to the dams. In a second study resistant dams were treated with an oral dose of 3.8 mg/kg under the same schedule as in the first study. In both studies the pups were 5 days old at the time of sacrifice. Control studies were run using 5 day old pups from non-dosed dams.

A third study employed the treatment of resistant and susceptible dams with endrin over a 2½ week period. Resistant dams were given ground feed which had been contaminated with 5 mg endrin per kg of feed. The contaminated feed was given every other day for 2½ weeks. Susceptible dams were given oral doses of endrin at the 0.51 mg/kg level every other day for 2½ weeks. Susceptible dams were dosed at this level because they were not able to survive the level of endrin in the feed which was given to the resistant dams. Pups from both strains were sacrificed at 2½ weeks of age and were analyzed for endrin content.

Extraction Procedure - The method of extraction employed mincing the pup in sodium sulfate (powdered) and extraction with purified redistilled petroleum ether.[1] Frozen resistant and susceptible pups were initially weighed and then rinsed with 5 ml of redistilled hexane. Three rinses were found to be sufficient to remove all exterior pesticide. Following the final rinse the frozen pups were minced using a scalpel and scissors in twice their weight of sodium sulfate. Mincing was continued until the sample was completely pulverized. At this point the sample was divided in half if the pup initially weighed more than six grams. The mixture was then quantitatively transferred into a 50 ml Sorvall omnimixer cup, and blended for five minutes at one half maximal speed. As a result of the mixing, the sample was more finely ground and more uniformly mixed. After mixing, the sample was transferred to a ground glass stoppered 50 ml centrifuge bottle and 20 ml of purified petroleum ether was added to the sample. The contents were then shaken for five minutes on a Burrel wrist action shaker and the petroleum ether phase was removed and filtered through filter paper on a Buchner funnel by air suction through a side armed Erlenmyer flask. Two additional extractions were made using 10 ml portions of purified petroleum ether. The entire contents of the 50 ml centirfuge bottle were then rinsed on to the

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[1]Roddy Young, Assistant Professor, Department of Biochemistry and Nutrition, VPI & SU, Blacksburg, Virginia. Personal Communication.

filter paper in the Buchner funnel and further rinsed with three 5 ml portions of petroleum ether. The petroleum ether collected was poured into a 50 ml beaker and evaporated to 10 ml under a stream of nitrogen gas. At this point the extract was ready to be cleaned of contaminating animal fats.

Clean up Procedure - Clean up of the petroleum ether extract was conducted according to the procedure developed by Wood (32) for the separation of chlorinated pesticides from contaminating fats. The procedure was designed for small samples ranging from two to five grams of tissue.

The petroleum ether extract was concentrated to 10 ml and 2.5 g of Celite 545 was added. The resulting slurry was heated at 60-70° under a gentle stream of N_2 gas. The slurry was constantly stirred with a glass rod until the petroleum ether had completely evaporated. All of the contents of the extract were absorbed by the Celite which was then used as a column packing by pouring into a glass column and packing tightly. At the top of the glass column a screw on top allowed for the application of air pressure and was free flowing in that there was no stopcock on the column. A second column which had a reservoir and a stopcock to adjust flow rate was also used. This column was made by allowing five grams of Florisil with 15% water by weight to settle into a hexane filled column. The sides of the column were constantly tapped in order for the Florisil to settle evenly.

After the Florisil settled, the hexane was allowed to flow out of the column until the hexane had just barely soaked into the top of the Florisil packing. The Celite column was then placed inside the Florisil column with the tip of the Celite column resting a few centimeters above the top of the Florisil packing. Six ml of DMSO was then added to the Celite and air pressure was applied to force the DMSO through the Celite. Two ml of the DMSO eluant was allowed to collect on top of the Florisil after which the air pressure was cut off and the Celite column was removed from the Florisil column.

After the removal of the Celite column, the stopcock on the Florisil column was opened until the DMSO had just soaked into the top of the Florisil. Redistilled hexane was then added to the Florisil column and was eluted at a flow rate of one drop per second. A total of 45 ml of the hexane eluant was collected and then evaporated to dryness under a gentle stream of N_2 gas. Appropriate volumes of hexane were added back to the sample prior to gas chromatographic analysis.

Gas Liquid Chromatography (GLC) - All GLC analysis was conducted using a Micro Tek MT 220 gas chromatograph equipped with a ^{63}Ni electron capture detector. The columns used were 92 cm long with a 3.2 mm i.d. and were packed with either a 3% OV-17 on 80/100 mesh Supelcoport packing or a 1% OV-225 on 80/100 mesh Supelcoport packing. The conditions used were as follows: 225° inlet temperature, 190° column temperature, 315° detector temperature, N_2 carrier

gas with a flow rate of 80 ml/min at 40 p.s.i. and electron power supply at 38 volts.

MFO Activity Studies - The demethylation of ethylmorphine and hydroxylation of aniline were used to determine the level of hepatic MFO activity and in both instances maximal activities were determined. Initially control levels of activity were determined for 2½ week old resistant and susceptible pups and resistant and susceptible adults (3-4 mo.).

In studying the effect of endrin, transferred via the milk, on the MFO activity in the pups, resistant and susceptible dams were dosed for 2½ weeks using feed containing 5 ppm endrin and oral doses at the 0.51 mg/kg level respectively. The dosing routine was identical to the dosing routine used to quantitate the amount of endrin passed to the pups in the milk of endrin treated dams over a 2½ week period. As was mentioned before, the susceptible dams were dosed differently than resistant dams because several of the susceptible dams died at the 5 ppm endrin level in the feed.

Hepatic MFO studies were also conducted on mature (3-4 mo.) resistant and susceptible animals dosed orally with endrin. Both groups received a single oral dose of endrin in corn oil at the 0.51 mg/kg level and were sacrificed 24 hr later. The 0.51 mg/kg level was chosen because it has been shown to be sub-lethal to the susceptible strain and although sub-lethal, it should nevertheless be stressful to the susceptible animals. Equal amounts were given

to both strains so that changes in activity in the susceptible animals could be directly compared to changes in the resistant activity levels.

Preparation of the Microsomal Fraction - Microsomes were isolated according to the procedure of Davies and co-workers (33) with modifications as described by Mgbodile and co-workers (34). Adult and immature pine mice were decapitated and exsanguinated. The livers were immediately removed, weighed, and placed in 1.15% KCl at 0-4°. In the case of 2½ week old pups two livers were pooled in order to have sufficient microsomal protein to carry out the assays. All further manipulations were conducted in solutions maintained at 0-4°.

The freshly excised liver was minced into small pieces and the KCl was decanted. The liver slices were washed 2 more times with cold 1.15% KCl. Homogenization of the liver was carried out in four volumes of cold 1.15% KCl per g of liver using a motor driven Potter-Elvehjem homogenizer. Homogenization was completed after six complete vertical strokes of the homogenizer. The resulting crude liver homogenate was then centrifuged for 20 min at 9,000 x g and the resulting supernatant was centrifuged for 75 min at 30,000 rpm using a type 30 rotor in a Beckman L2-65B preparative ultracentrifuge. After centrifugation was completed, the supernatant was discarded and the microsomal pellet was resuspended in cold 0.02M Tris buffer in 1.15% KCl (pH 7.4). The resuspension was accomplished using a motor driven Potter-Elvejhem homogenizer with a five

ml capacity. Six complete vertical strokes were used to resuspend the microsomal pellet. Care was taken with the volume of buffer used to homogenize the microsomal pellet so as not to over-dilute the microsomal protein.

Enzyme Assay Conditions - Assay conditions were essentially as those described by Gram et al. (35). Certain modifications were developed to aid in the running of the assays. All assays were carried out in neoprene centrifuge tubes which were placed into 20 ml beakers half filled with water in a Dubnoff metabolic shaker set at 120 oscillations per minute with a constant temperature of 37°.

Initially the assay mixture minus the microsomal protein was preincubated for 15 minutes. The preincubation mixture contained 0.3 ml of 1.15% KCl, 0.3 ml of 120mM Tris buffer (pH 7.4), 0.2 ml NADPH generating system and 0.1 ml of substrate (aniline or ethylmorphine). Concentrations of the components of the NADPH generating system were as follows: $NADP^+$ 2mM, glucose-6-phosphate 50mM, $MgCl_2$ 5mM, and 10 to 20 units of glucose-6-phosphate dehydrogenase per ml. Both assays were initiated by the addition of 0.1 ml of microsomal suspension at a concentration of 6 to 12 mg microsomal protein per ml.

Ethylmorphine demethylase assays were run for ten min after the addition of the microsomal protein while the aniline hydroxylation assays were run for 20 min. The assays were linear for protein concentration and time. Both reactions were stopped by the addition of 1 ml of 20% trichloroacetic acid (36) and subsequent submersion in

ice. The centrifuge tubes containing the percipitated protein were placed in a Sorvall SM-24 rotor and centrifuged at 10,000 rpm for 10 min so as to form a pellet of percipitated protein. The supernatant was carefully decanted into small test tubes.

Determination of Products - Formaldehyde was assayed by the procedure as developed by Nash (37) and further modified by Davies (33). The procedure for the determination of p-aminophenol was developed by Guarino (38).

In the case of formaldehyde determination 1 ml of the supernatant from the TCA stopped reaction was combined with 1 ml of the Nash reagent. The color was developed for one hour in the dark and absorbance readings were then taken at 412 nm. The Nash reagent consisted of 75 g of ammonium acetate, 250 ml of distilled water, 1 ml of acetyl acetone and 1.5 ml of acetic acid. Fresh Nash reagent was made one day prior to each experiment.

P-aminophenol was determined by adding 2.5 ml of the phenol reagent to 0.5 ml of the supernatant and allowing the color to develop for 30 min in the dark. Absorbance was then read at 620 nm. The phenol reagent was made by adding 26.5 g of tribasic potassium phosphate to 250 ml of 1% aqueous phenol. The phenol reagent was also made fresh before each experiment.

It should be noted that both the color reaction for formaldehyde and p-aminophenol determinations tended to change absorbance upon exposure to light and with time. Therefore, each

sample in a group of samples was staggered by 20 sec and maintained in the dark until just prior to reading.

Statistical Analysis - Paired t-tests were used to determine significance between averages at the .05 level of probability for all of the data on endrin and DDE content. A three way analysis of variance was used to determine significant differences for the ethylmorphine demethylase and aniline hydroxylase activities. The effects of strains, age and treatment were all considered to be fixed effects.

RESULTS

Efficiency of the Extraction and Clean up Procedures - The data from Table I show that the clean up procedure yielded approximately 100% of the endrin added to the Celite. All of the endrin was eluted within the first 40 ml of hexane eluant, which compares favorably with the initial work done to develop this procedure (32). Table II shows the results of the determination of the per cent recovery of the combined extraction and clean up procedures. Known amounts of endrin were given to live pups by an intraperitoneal injection with a microliter syringe. Approximately 83% of the administered dose was recovered by the extraction and clean up procedures. The data also indicate good consistency from one extraction to another.

Endrin Content in the Pups - Figure 1 compares gas chromatograms of resistant and susceptible pups from control dams with a gas chromatogram of endrin. Direct comparison gives no indication of any peak from the control pups which might interfere with the determination of endrin. The chromatogram for the resistant pup is representative of four control extractions in which no endrin was detected.

In making the quantitative determination of endrin in the pups by gas chromatography each sample injection from the pup extract was preceded by the injection of an endrin standard. A ratio of the detector response was made between the known amount in the standard to the unknown amount of endrin present in the extract. Care was

Table I

Per cent recovery of the clean up procedure for endrin

Experiment	Fraction number[1] 1	2	3	4	Total Recovered	Amount Added	Per cent Recovery
I	80.40	17.20	0.80	-	98.40	102.00	98
II	0.80	1.34	0.08	-	2.22	2.20	101
III	1.70	1.85	0.60	-	3.72	3.70	101

[1] µg/15 ml of the hexane eluant.

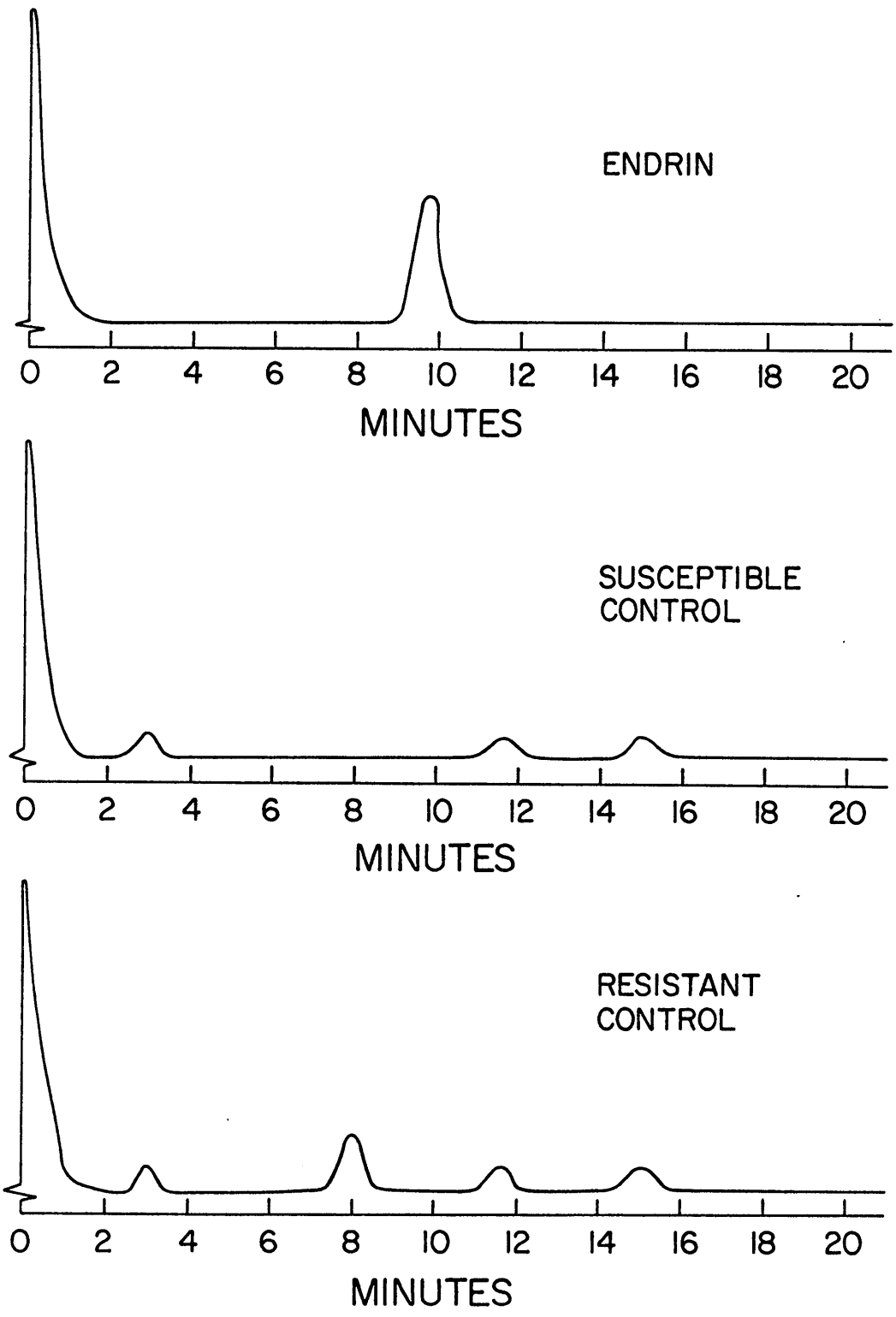
TABLE II

Per cent recovery of endrin administered to the pups

Experiment	Endrin injected (µg)[1]	Endrin Recovered (µg)	Per cent Recovery
I	2.50	2.02	80.80
II	2.50	2.12	84.80
III	2.50	2.07	82.80

[1]Endrin administered i.p. to pups.

Figure 1. Reconstructed gas chromatograms of endrin, extract from susceptible control and an extract from resistant control. GLC conditions were those as outlined in the Methods section.


ENDRIN
0 2 4 6 8 10 12 14 16 18 20
MINUTES
SUSCEPTIBLE
CONTROL
0 2 4 6 8 10 12 14 16 18 20
MINUTES
RESISTANT
CONTROL
0 2 4 6 8 10 12 14 16 18 20
MINUTES

taken to make sample and standard injections fall within the linear range of detector response for endrin.

Endrin content in pups from endrin dosed dams is given in Table III. Equivalent amounts of endrin are present in resistant and susceptible pups from dams receiving the oral 0.51 mg/kg dose level of endrin for three days. About 0.3 µg of endrin was present in pups of both strains at the time of sacrifice. This Table shows that there is no difference in the amounts of endrin transferred via the milk if dams of both strains are given equal doses.

Resistant dams were also dosed at the 3.8 mg/kg level because it was equitoxic to the 0.51 mg/kg level given to susceptible dams (38). The resistant pups from dams dosed at the 3.8 mg/kg level contained about four times the amount of endrin as did pups dosed at the 0.51 mg/kg level. There is, however, a decrease in the per cent of the total dose given to the dam which was present in the pup. A value of 0.27% was obtained for the 3.8 mg/kg group as compared to a value of 0.62% for the 0.51 mg/kg group. These data suggest that a point can be reached with resistant dams where an increase in the amount of endrin given to the dam will not result in an increase in the amount passed to the suckling pup.

Endrin content in pups from dams dosed over a period of 2½ weeks with endrin was found to be similar for both strains. If the data is expressed in terms of µg of endrin per g of body weight a value of 0.05 µg/g is seen for both resistant and susceptible pups. The microsomal studies that were performed used pups from dams

TABLE III

Whole body endrin content of pine mouse pups[1]

	5 Day			2½ weeks	
	Res.[2]	Res.[3]	Sus.[2]	Res.[4]	Sus.[2]
µg endrin	0.32 ± .04* (4)	1.42 ± .20 (4)	0.31 ± .11* (5)	0.44 ± .07 (4)	0.36 ± .02 (2)
µg/g body wt.	0.08 ± .01*	0.35 ± .04	0.09 ± .02*	0.05 ± .01	0.05 ± .01
Per cent of total dose to dam	0.62 ± .05*	0.27 ± .06	0.64 ± .23	0.21 ± .02	0.21 ± .02

[1]Averages ± S.E.M., numbers in parentheses indicate animals per group.

[2]From dams given oral 0.51 mg/kg doses of endrin.

[3]From dams given oral 3.8 mg/kg doses of endrin.

[4]From dams maintained on 5 mg/kg endrin in diet.

*Significantly different ($P < 0.05$) from Res. 3.8 mg/kg group.

dosed at the 5 ppm dietary level, in the case of resistant dams and 0.51 mg/kg oral doses for the susceptible dams. In order for a direct comparison of activities to be made both strains of pups should contain similar amounts of endrin.

The data also indicate the fact that little accumulation of endrin by the pups occurs. Approximately 0.4 µg was present in pups from dams dosed over a 2½ week period while approximately 0.3 µg was present in pups from dams dosed for three days at the 0.51 mg/kg oral dose.

Presence of DDE in Resistant Pups - In comparing gas chromatograms of resistant and susceptible pups, a substantial peak preceding the endrin peak was found in extracts from resistant pups but was barely detectable but not quantitated in extracts from susceptible pups. This relationship persisted throughout all of the extracts examined. Retention values were measured by finding the distance from the solvent peak to the peaks of various pesticides. Retention values were then made relative to an internal endrin standard. Table IV presents the relative retention values for the unknown, dieldrin and DDE peaks. The unknown appears to be DDE when a comparison of the relative value of 0.834 for the unknown is made to the relative value of 0.831 for DDE. Figure 2 compares a chromatogram of an extract of a resistant pup to a chromatogram of the same extract which had been spiked with DDE. These chromatograms show that DDE has exactly the same retention time as does the suspected DDE peak. Levels of DDT and DDE have been found in the laboratory

TABLE IV

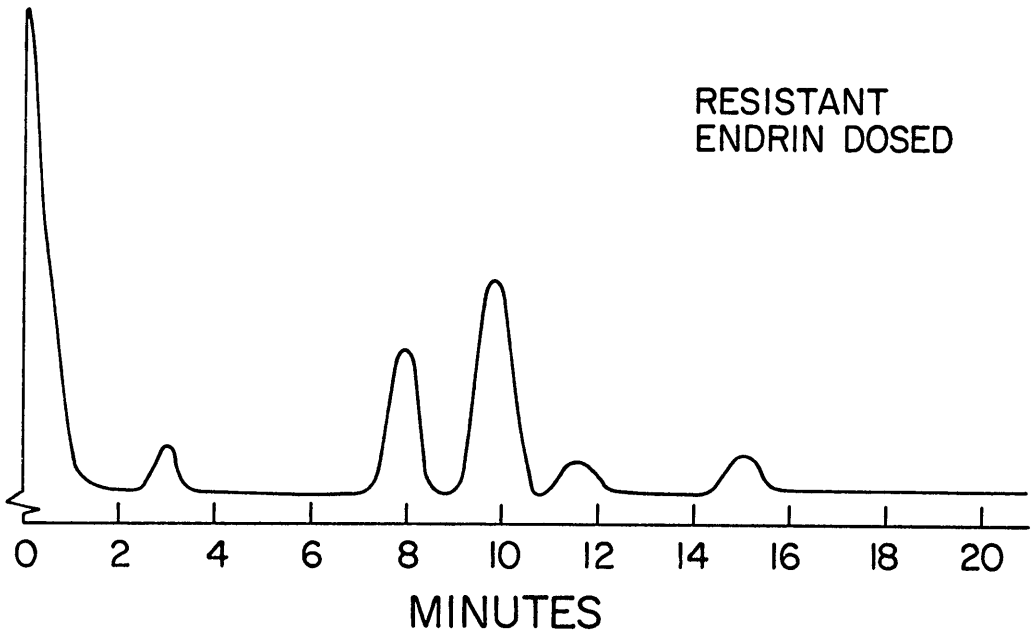
Relative retention values of DDE, dieldrin and the unknown peak as compared to endrin[1]

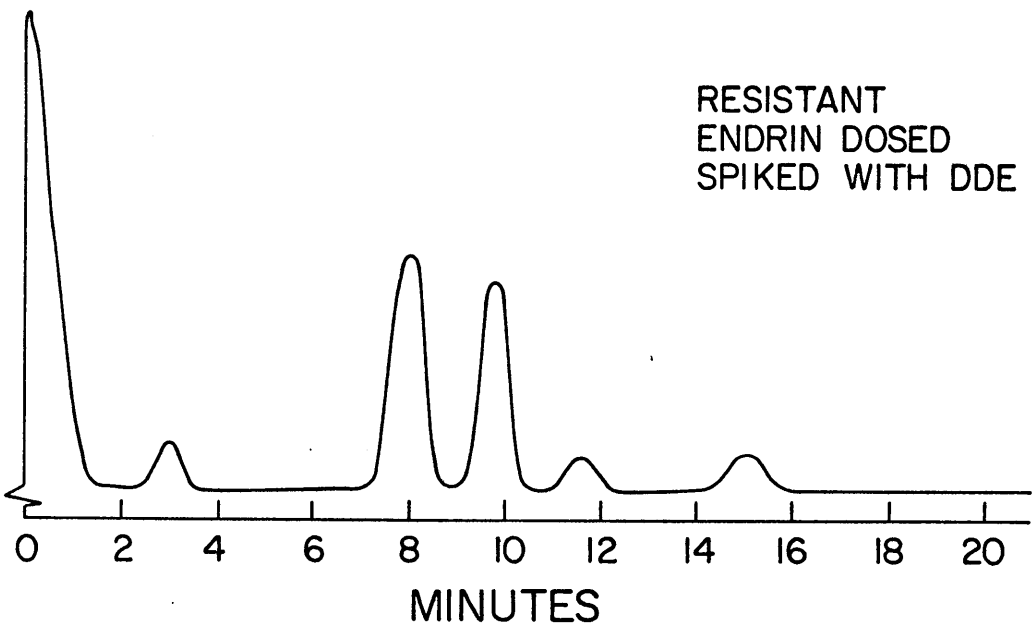
	Relative retention values[2]		
Experiment	Dieldrin	Unknown	DDE
I	0.802	0.839	0.840
II	0.797	0.835	0.830
III	0.810	0.834	0.824
IV		0.834	
V		0.830	
VI	———	0.830	———
Average	0.803	0.834	0.831

[1]The following GLC conditions were used: 1% OV-225 on 80/100 mesh Supelcoport packing, 225° inlet temperature, 190° column temperature, 315° detector temperature and N_2 carrier gas with a flow of 80 ml/min at 40 p.s.i.

[2]Distance from solvent peak (cm) to pesticide peak divided by distance from solvent peak to endrin peak (chart rate was 1.27 cm per min).

Figure 2. Reconstructed gas chromatograms of an extract from a resistant pup from an endrin dosed dam and the same extract to which DDE had been added. GLC conditions were those as outlined in the Methods section.


RESISTANT
ENDRIN DOSED
0
2
4
6
8
10
12
14
16
18
20
MINUTES


RESISTANT
ENDRIN DOSED
SPIKED WITH DDE
0
2
4
6
8
10
12
14
16
18
20
MINUTES

feed in amounts less than 1 ppm.[1] Therefore it is not surprising that DDE be present in the pups but it is unusual for DDE to be found exclusively in resistant pups.

It should be mentioned, however, that although the indications are very strong that the unknown peak is in fact DDE, conclusive proof would require mass spectrum analysis. GLC analysis is sufficient for qualitative determination under strictly controlled conditions.

Table V presents the data on DDE content in the pups from control and endrin dosed dams. The limit of quantitation of DDE by the extraction, clean up and subsequent GLC analysis is approximately 0.01 µg per pup. All susceptible values are presented as being less than 0.01 µg. Five day old resistant pups from control dams contained a total of 0.054 µg of DDE per pup, which was less than half the amount in pups from resistant dams dosed at the 0.51 and 3.8 mg/kg oral level. DDE amounts are quite variable in 2½ week old resistant pups from either endrin treated or control dams. The values ranged from 0.2 to 27 µg.

Hepatic MFO Activity - Table VI contains the data for ethylmorphine demethylase and aniline hydroxylase maximal activities. The data were analyzed as a three way analysis of variance by considering age, strain and treatment as three fixed effects. In comparing mature versus immature animals for the two activities measured, it was found that the MFO activity of the mature animals

[1]Unpublished data.

TABLE V

DDE content in resistant and susceptible pups[1]

DDE	5 Day			2½ weeks	
	Res.	Res.[2]	Sus.	Res.	Sus.
Control					
Total µg	0.05 ± .004 (2)	-	<.01 (2)	0.20 to 21.90 (2)	<.01 (2)
µg/g body wt	0.01 ± .001	-	-	0.04 to 2.74	-
Endrin Dosed					
Total µg	0.18 ± .015[a,*] (4)	0.160 ± .005* (4)	<.01[a] (5)	0.12 to 27.30 (4)	<.01 (2)
µg/g body wt	0.04 ± .003*	0.040 ± .005*	-	0.01 to 2.88	-

[1]Average ± S.E.M., numbers in parentheses indicate the number of animals per group.

[2]Oral doses of endrin to the dam at the 3.80 mg/kg level.

[a]Oral doses of endrin to the dam at the 0.51 mg/kg level.

*Significantly different ($P < 0.05$) from the 5 day res. control using paired t-tests.

TABLE VI

Hepatic microsomal activity of mature and 2 week old pine mice - maximal activity determinations[1]

Group	Ethylmorphine demethylase[2]		Aniline hydroxylase[3]	
	Control[4]	Endrin dosed[4]	Control[4]	Endrin dosed[4]
Mature res.	1497 ± 182[5] (6)	1198 ± 124[5] (6)	217 ± 13	189 ± 18
Mature sus.	1359 ± 72[5] (4)	800 ± 44[5] (6)	205 ± 10	156 ± 13
2 week res.	1509 ± 126[5] (7)	1049 ± 62*[5] (4)	222 ± 9	142 ± 15*
2 week sus.	1312 ± 83[5] (5)	945 ± 148*[5] (4)	180 ± 27	177 ± 19*

[1]Average ± S.E.M., numbers in parentheses indicate the animals per group.

[2]nmoles formaldehyde per mg microsomal protein per hour.

[3]nmoles p-aminophenol per mg microsomal protein per hour.

*Pups were from endrin dosed dams as outlined in Methods.

[4]Significant treatment effect at $P < 0.05$ using a three way analysis of variance.

[5]Significant strain effect at $P < 0.05$ using a three way analysis of variance.

did not differ from the activity of the immature animals. The average values for the ethylmorphine demethylase activities are equivalent for control mature and immature resistant animals and for control mature and immature susceptible animals.

A significant difference was observed ($P < 0.05$) in comparing ethylmorphine demethylase activity between strains. No significant difference was observed between strains for aniline hydroxylase activity. In the case of ethylmorphine demethylase activity resistant activities were greater in each comparison with a per cent difference range from 9 to 33%.

A significant difference ($P < 0.05$) was found for both activities in comparing endrin treated versus control animals. As can be seen from Table VI, all of the control activities were greater than the corresponding endrin treated activities. The per cent differences were found to be greatest for ethylmorphine demethylase activity between mature susceptible control and endrin dosed animals and for aniline hydroxylase activity between immature resistant control and endrin dosed animals. Endrin, therefore, appears to cause a significant decrease in MFO activity in the pine mouse as can be determined by aniline hydroxylase and ethylmorphine demethylase activities.

DISCUSSION

The initial question to be resolved dealt with whether the tolerance observed in the resistant strain of pine mice was due in part to an acquired trait. Previous laboratory studies (5,31) indicated that resistant animals raised under pesticide free conditions showed a higher LD_{50} value and higher benzpyrene hydroxylase activity than did susceptible animals. The animals used in these studies were one generation removed from the orchard. These data plus the intermediate LD_{50} value for progeny of resistant crossed with susceptible animals (5) gives a very strong case for an inherited resistance.

The possibility existed, however, that endrin was stored by the orchard animals and that lactating females might pass enough endrin to their suckling pups to develop an acquired resistance even under pesticide free conditions. It could be implied, therefore, that the data obtained from the progeny of orchard animals could reflect endrin, even in small amounts, as being a powerful inducer of hepatic MFO activity. It might then increase microsomal activity sufficiently so as to put the animals in a tolerant state against endrin.

The results of the extraction studies alone discredit the validity of thinking that residual endrin causes a tolerant condition toward endrin in the offspring. No measurable endrin was detected in resistant pups from control dams. Residual endrin therefore,

cannot be the cause of the persistence of the resistant state. These data are even more convincing when it is considered that the dams used in the breeding pairs were survivors of a 20 mg/kg oral dose of endrin.

The rest of the data on the extraction of endrin from the pups also yields interesting information. Dosed at equal levels of endrin, both resistant and susceptible dams pass equal amounts of endrin to their offspring. This indicates that there is no difference in the incorporation of endrin into the milk of dams of either strain. It can also be seen that resistant pups from dams dosed at the 3.8 mg/kg level contain substantially more endrin than do pups of either strain dosed at the 0.51 mg/kg level. There is, however, a significant decrease in the per cent of the total amount given to the dam which is found in the pup. This suggests that a level can be reached in dosing the resistant dam where any further increase in the amount given would not result in an increase in the amount of endrin found in the pup. Previous LD_{50} values (5) indicate that this could not be the case for susceptible dams because they would not be able to survive a dose much greater than a single 3.8 mg/kg oral dose.

Data from pups of dams dosed over a 2½ week period reveal that endrin is not accumulated by the pups to any appreciable degree. This can be seen by comparing the microgram quantities of endrin present in resistant and susceptible pups from dams dosed for three days at the 0.51 mg/kg level. The data also show that

although resistant dams received more endrin over the 2½ week period than did susceptible dams, the quantities of endrin, when expressed as micrograms of endrin per gram of body weight, present in pups of both strains have the same value. This was important because the amounts of endrin in the pups should be equal if comparisons of the effects of endrin on the MFO activity are to be made.

An important, although unexpected, result of the extraction studies was the presence of DDE in resistant pups whereas only trace amounts were found in susceptible pups. Although it is not known exactly how much DDT and DDE each dam consumed, it was interesting to note how consistent the DDE data was for 5 day old resistant pups from either endrin dosed or control dams. There is also a significant two fold increase in the amounts of DDE found in pups from endrin dosed resistant dams as compared to the amounts in pups from control resistant dams.

It is not possible from this present study to determine why DDE appears in extracts from resistant pups whereas only trace amounts are present in extracts from susceptible pups. There are several possible explanations such as a difference in the ability of resistant and susceptible dams to incorporate DDE into the milk, greater metabolism of DDT to DDE by resistant dams, greater metabolism of DDT and DDE by resistant pups, or a greater accumulation of DDE by resistant dams. Regardless of the real cause of this phenomenon it represents a real difference between the two strains and needs to be researched to a greater extent.

In looking at the MFO data it is useful to compare the MFO activities of the immature (2½ week) to mature (3-4 mo.) pine mice. Regardless of strain or treatment it is evident that immature pine mice have ethylmorphine demethylase and aniline hydroxylase activities equal to that of mature pine mice. This in direct contrast to papers reviewed (15,16,18,31) which indicate that the earliest time at which MFO activity reaches its peak is 30 days after birth. The only activity reviewed that reaches an earlier peak is biphenyl-2-hydroxylase (16). Recent work by Fouts and Devereux (40) indicates that benzpyrene hydroxylase activity reaches a maximum at 30 days of age in rabbits. Pine mice are unique from other species which have been studied and yet even with the pine mouse there is a conflict as to when MFO activity reaches its highest level. Hartgrove and Webb (31) have shown that benzpyrene hydroxylase activity does not reach its peak until 6 weeks of age. This information indicates that MFO activities may increase at different rates with age and where one activity may reach its peak at 6 weeks another may peak at 2½ weeks. This rationale is supported by the work of Basu, Dickerson and Parke (16) which demonstrated with neonatal rats that biphenyl-2-hydroxylase activity peaked at the age of 20 days while that of p-nitrobenzoate reductase peaked at 40 days.

An important question that has not been resolved relates to the MFO capabilities of immature and mature pine mice in regard to endrin metabolism. The MFO activity as determined by ethylmorphine

demethylase and aniline hydroxylase activities may only be reflections of a partial picture of MFO activity in pine mice.

A strain difference was found for ethylmorphine demethylase activity whereas no difference between strains was found for aniline hydroxylase activity. Hartgrove and Webb (31) observed a 2½ fold higher benzpyrene hydroxylase activity for resistant as compared to susceptible pine mice. Although the resistant ethylmorphine demethylase activity was significantly higher than the susceptible activity the individual differences ranged from 9 to 33% which is far below the 2½ fold difference in activity mentioned above.

This comparison of activities reflects an important consideration in the interpretation of MFO activity data, which is that a single activity may not be sufficient to categorize MFO activity of a given species or strain of a species. Work by Furner and coworkers (41) has shown differences between rat strains in MFO activity to not only be dependent upon the strain but also upon the activity assayed. In comparing p-nitroanisole metabolism, rats of the Wistar strain had a three fold higher activity than did rats of the Holtzman strain. However, no difference was noted between the two strains when hexobarbital metabolism was assayed. Possibly this same situation holds true for the resistant and susceptible pine mice in that benzpyrene hydroxylation would more effectively reflect differences in the ability of the two strains to detoxify endrin.

The effect of endrin dosing both directly to mature pine mice and indirectly, via the milk, to immature pine mice is an example of the failure of a potential inducing agent to induce MFO activity. Regardless of strain or age, endrin treatment reduces the aniline hydroxylase and ethylmorphine demethylase activities below that of control levels. The greatest differences seen are approximately 60% with ethylmorphine demethylase activity for the mature susceptible dosed versus control animals and with aniline hydroxylase activity for immature resistant dosed versus control animals. Bunyan and Page (27) found similar results when they treated rats with DDMU (a metabolite of DDT) and observed a decrease from control activity for ethylmorphine demethylase activity. Davison and Sell (29) also reported decreases in aniline hydroxylase activities in chickens after treatment with DDT.

In a study similar to this paper Greene and co-workers (24) have demonstrated that perinatal rats exposed to dieldrin show an increase in cytochrome P-450 levels and ethylmorphine demethylase activity. So in diverging ways both studies have shown that MFO activity can be altered by the transferral of chlorinated pesticides in the milk to the perinatal offspring.

Work of this type provides information of importance toward revealing what effects foreign compounds have upon the very young. Especially important are the effects that compounds such as drugs, pesticides and carcinogens transferred via the milk have on the hepatic MFO activity of the neonate.

SUMMARY

Endrin was shown to be transferred via the milk to suckling pine mouse pups. Resistant and susceptible dams transferred equal amounts of endrin provided that dams of both strains were dosed with equal amounts of endrin. Endrin was not accumulated by the pups of either strain when the amounts of endrin present in the pups over the 2½ week dosing schedule were compared to the amounts present over a 5 day dosing schedule.

Unexpectedly, DDE was found to be present exclusively in extracts from resistant pups. Susceptible pups contained only trace amounts which could not be quantitated adequately by GLC determination. It was also noted that resistant pups from endrin dosed dams for the 5 day dosing schedule had over twice as much DDE as did resistant pups from control dams.

Hepatic MFO activity as measured by the maximal activities for the demethylation of ethylmorphine and the hydroxylation of aniline was shown to be equivalent for the comparison between age groups (mature vs. immature). Ethylmorphine demethylase activity was shown to be significantly higher for the resistant as compared to the susceptible strain, however, there was no significant difference noted for aniline hydroxylase activity. A significant decrease in both activities was noted for the comparison between endrin dosed and control animals.

References

1. Boyle, C. M. (1960) Case of apparent resistance of Rattus norvegicus Berkenhout to anticoagulant poisons. Nature, 188, 517.

2. Ozburn, G. W. and Morrison, F. (1962) Development of a DDT tolerant strain of laboratory mice. Nature, 196, 1009-1010.

3. Gutherie, F. E., Monroe, R. J. and Abernathy, C. O. (1971) Response of the laboratory mouse to selection for resistance to insecticides. Toxicol. Appl. Pharmacol. 18, 92-101.

4. Webb, R. E. and Horsfall, F. (1967) Endrin resistance in the pine mouse. Science, 156, 1762.

5. Webb, R. E., Hartgrove, R. W., Randolph, W. C., Petrella, V. J. and Horsfall, F. (1973) Toxicity studies in endrin-susceptible and resistant strains of pine mice. Toxicol. Appl. Pharmacol. 25, 42-47.

6. Venkatesan, N., Arcos, J. C. and Argus, M. F. (1971) Induction and repression of microsomal drug-metabolizing enzymes by polycyclic hydrocarbons and phenobarbital: theoretical models. J. Theor. Biol. 33, 517-537.

7. Conney, A. H., Welch, R., Kurtzman, R., Chang, R., Jacobson, M., Munro-Faure, A. D., Peck, A. W., Bye, A., Poland, A., Poppers, P. J., Finster, M. and Wolff, J. A. (1971) Effects of environmental chemicals on the metabolism of drugs, carcinogens and normal body constituents in man. Annals N. Y. Acad. Sci. 179, 155-172.

8. Mannering, G. J. (1971) Properties of cytochrome P-450 as affected by environmental factors: qualitative changes due to administration of polycyclic hydrocarbons. Metabolism, 20, 228-245.

9. Estabrook, R. W., Franklin, M. R., Cohen, B., Shigamatzu, A. and Hildebrandt, A. (1971) Influence of hepatic microsomal mixed function oxidation reactions on cellular metabolic control. Metabolism, 20, 187-199.

10. Remmer, H. (1972) The induction of the enzymic "detoxication" system in liver cells. Rev. Can. Biol. 31, 193-222.

11. Gillette, J. R., Davis, D. C. and Sasame, H. A. (1972) Cytochrome P-450 and its role in drug metabolism. Ann. Rev. Pharmacol. 12, 57-84.

12. Hart, L. G. and Fouts, J. R. (1965) Studies on the possible mechanisms by which chlordane stimulates hepatic microsomal drug metabolism in the rat. Biochem. Pharmacol. 14, 263-272.

13. Darby, F. J. (1971) Changes in drug-metabolizing activities in the livers of suckling rats as a result of treatment of lactating mothers with phenobarbitone and chlorpromazine. Biochem. J. 122, 41-47.

14. Hart, L. G., Adamson, R. H., Dixon, R. L. and Fouts, J. R. (1962) Stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. J. Pharmacol. Exp. Ther. 137, 103-106.

15. Henderson, P. Th. (1971) Metabolism of drugs in rat liver during the perinatal period. Biochem. Pharmacol. 2, 793-805.

16. Basu, T. K., Dickerson, J. W. T. and Parke, D. V. W. (1971) Effect of development on the activity of microsomal drug-metabolizing enzymes in rat liver. Biochem. J. 124, 19-24.

17. Short, C. R. and Stith, R. D. (1973) Perinatal development of hepatic microsomal activity in swine. Biochem. Pharmacol. 22, 1309-1319.

18. MacLeod, S. M., Renton, K. W. and Eade, N. R. (1972) Development of hepatic microsomal drug-metabolizing enzymes in immature male and female rats. J. Pharmacol. Exp. Ther. 183, 489-498.

19. Uehleke, H., Reiner, O. and Hellmer, K. H. (1971) Perinatal development of tertiary amine N-oxidation and NADPH cytochrome c reduction in rat liver microsomes. Res. Commun. Chem. Path. Pharmacol. 2, 793-805.

20. Conney, A. H. and Burns, J. J. (1972) Metabolic interactions among environmental chemicals and drugs. Science, 178, 576-585.

21. Kroger, M. (1972) Insecticide content in human milk. J. Pediatrics, 80, 401-405.

22. Woolley, D. E. and Talens, G. M. (1971) Distribution of DDT, DDD and DDE in tissues of neonatal rats and in milk and other tissues of mother rats chronically exposed to DDT. Toxicol. Appl. Pharmacol. 18, 907-916.

23. Ottoboni, A. and Ferguson, J. I. (1969) Excretion of DDT compounds in rat milk. Toxicol. Appl. Pharmacol. 15, 56-61.

24. Greene, F. E., Stevens, J. T., Soliman, M. R. I. and Oberholser, K. A. (1974) Effects of perinatal dieldrin exposure on hepatic microsomal enzymes of immature and adult rats (Abstract). Toxicol. Appl. Pharmacol. 29, 128.

25. Hart, L. G. and Fouts, J. R. (1963) Effects of acute and chronic DDT administration on hepatic microsomal drug metabolism in the rat. Proc. Soc. Exp. Biol. 114, 228-292.

26. Chhabra, R. S. and Fouts, J. R. (1973) Stimulation of hepatic microsomal drug-metabolizing enzymes in mice by 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 3,4-Benzpyrene. Toxicol. Appl. Pharmacol. 25, 60-70.

27. Bunyan, P. J. and Page, J. M. (1973) Pesticide-induced changes in hepatic microsomal enzyme systems. Some effects of 1,1-di(p-chlorophenyl)-2,2-dichloro-ethylene (DDE) and 1,1-di(p-chlorophenyl)-2-chloroethylene (DDMU) in the rat and Japanese Quail. Chem.-Biol. Interactions, 6, 249-257.

28. Abernathy, C. O., Hodgson, E. and Guthrie, F. E. (1971) Structure-activity relationships on the induction of hepatic microsomal enzymes in the mouse by 1,1,1-trichloro-2,2-bis(p-chlorophenylethane(DDT) analogs. Biochem. Pharmacol. 20, 2385-2393.

29. Davison, K. L. and Sell, J. L. (1972) Dieldrin and p,p'-DDT effects on some microsomal enzymes of livers of chickens and mallard ducks. J. Agri. Food Chem. 20, 1198-1205.

30. Webb, R. E., Randolph, W. C. and Horsfall, F. (1972) Hepatic benzpyrene hydroxylase activity in endrin susceptible and resistant pine mice. Life Sciences, 11, 477-481.

31. Hartgrove, R. W. and Webb, R. E. (1973) The development of benzpyrene hydroxylase activity in endrin susceptible and resistant pine mice. Pest. Biochem. Physiol. 3, 61-65.

32. Wood, N. F. (1969) Extraction and clean up of organochlorine pesticide residues by column chromatography. Analyst, 94, 399-405.

33. Davies, D. S., Gigon, P. L. and Gillette, J. R. (1968) Sex differences in the kinetic constants for the N-demethylation of ethylmorphine by rat liver microsomes. Biochem. Pharmacol. 17, 1865-1872.

34. Mgbodile, M. U. K., Hayes, J. R. and Campbell, T. C. (1973) Effect of protein deficiency on the inducibility of the hepatic microsomal drug-metabolizing enzyme system - II. Biochem. Pharmacol. 22, 1125-1132.

35. Gram, T. E., Guarino, A. M., Schroeder, D. H. and Gillette, J. R. (1969) Changes in certain kinetic properties of hepatic microsomal aniline hydroxylase and ethylmorphine demethylase associated with postnatal development and maturation in male rats. Biochem. J. 113, 681-685.

36. Barker, E. A. and Smuckler, E. A. (1972) Altered microsome function during acute thioacetamide poisoning. Mol. Pharmacol. 8, 318-326.

37. Nash, T. (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55, 416-421.

38. Guarino, A. M., Gram, T. E., Gigon, P. L., Greene, F. E. and Gillette, J. R. (1969) Changes in Michaelis and spectral constants for aniline in hepatic microsomes from phenobarbital-treated rats. Mol. Pharmacol. 5, 131-136.

39. Petrella, V. J. (1973) Metabolism studies of endrin in endrin-susceptible and resistant pine mice. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. pp. 25.

40. Fouts, J. R. and Devereux, T. R. (1972) Developmental aspects of hepatic and extrahepatic drug-metabolizing enzyme systems: microsomal enzymes and components in rabbit liver and lung during the first month of life. J. Pharmacol. Exp. Ther. 183, 458-468.

41. Furner, R. L., Gram, T. E. and Stitzel, R. E. (1969) The influence of age, sex and drug treatment on microsomal drug metabolism in four rat strains. Biochem. Pharmacol. 18, 1135-1641.

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THE TRANSFER OF ENDRIN VIA THE MILK TO PINE MOUSE PUPS AND THE RESULTANT EFFECT ON HEPATIC MICROSOMAL ACTIVITY

by

Stephen Gilbert Hundley

(ABSTRACT)

Many lipophilic pesticides are known to be transferred to offspring via the mother's milk. The present study was conducted to determine how much endrin was transferred from endrin resistant and susceptible dams to their suckling pups and to further characterize the effects that endrin may have on the hepatic mixed function oxidase (MFO) system in the pups.

Dosing of the dams with endrin began one day after birth with either (1) oral doses of endrin in corn oil or (2) a mixture of endrin in ground feed. The total amount of endrin in the pup was determined by gas chromatography. MFO activity was determined in 2½ week old pups and for adult animals using maximal activities for the demethylation of ethylmorphine and hydroxylation of aniline.

No difference in the amount of endrin present in the pups was observed between strains provided both received equal amounts of endrin. MFO activity for endrin dosed mature animals and for 2½ week old pups from endrin dosed dams exhibited a significant decrease from control activities. There was no difference in MFO activities between age groups. A significantly higher ethylmorphine

demethylase activity was observed in comparing the resistant to the susceptible strain but there was no significant difference in aniline hydroxylase activity.