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Background studies on the effects of DDT on members of inbred strains and of natural populations of Mus musculus.

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BACKGROUND STUDIES ON THE EFFECTS OF DDT ON MEMBERS OF INBRED STRAINS AND OF NATURAL POPULATIONS OF *Mus musculus*

BY

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ABSTRACT

Two inbred strains of the house mouse, Mus musculus, and a sample of wild mice were (a) tested with p,p'-DDT (1,1-Bis-(p-chlorophenyl)-2,2,2-trichloroethane) to determine the LD_{50}, and (b) examined for metabolic rate. Also several enzyme systems were examined for possible inhibition by p,p'-DDT. The purpose of the study was to gain basic information necessary for the investigation of the effects of pesticides on natural populations of the house mouse.

The LD_{50} of p,p'-DDT in C3HeB/FeJ was determined as approximately 730-740 mg/kg. For C57BL/10J the established LD_{50} is approximately 705-715 mg/kg. The lower values are based on probit analysis and the higher on Reed-Muench analysis of data. The difference between the estimated LD_{50}'s of the two strains was considered not to be the result of inherited differences in tolerance. These values form a useful baseline for comparison with natural populations. Preliminary evidence does not indicate that the general level of tolerance to p,p'-DDT is different between in wild and laboratory mice.

No changes were found in the patterns of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, or cytoplasmic $NADP^+$-isocitrate dehydrogenase activity after starch gel electrophoresis of kidney extracts from mice injected with p,p'-DDT at doses of 600-800 mg/kg. Cytoplasmic $NADP^+$-malate dehydrogenase (malic enzyme) activity is reduced or absent in extracts of the kidneys and livers of mice in convulsions or dead because of p,p'-DDT intoxication. Since there is no evidence of direct

inhibition of malic enzyme by p,p'-DDT in concentrations up to 90 ppm, the reduction in malate dehydrogenase activity is probably the result of the fasting state brought about by convulsions.

The oxygen consumption of females of two inbred strains at 6-8 weeks of age (strains C3HeB and C57BL) was found to be 64.2 and 66.2 ul/g/min respectively. A group of ten female wild mice (7-9 weeks old) had an oxygen consumption of 86.6 ul/g/min, significantly higher than either inbred strain.

The use of natural populations of Mus musculus is suggested in order to evaluate the effects of environmental agents such as pesticides. This would provide information on mammals in their natural state, permit comparisons with laboratory mice, test conclusions reached in studies with laboratory mice, and may provide some information on the evolutionary significance of some pesticides.

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CHAPTER I

INTRODUCTION

As a research mammal, the house mouse, Mus musculus, has several distinct advantages, among which are relative ease of maintenance, short generation time and the availability of a variety of well defined inbred strains.

Recently Palmes and Pup (1970) have proposed that the mouse would be an excellent model mammal to examine the effects of "environmental agents". They furthermore suggest a dihybrid cross of inbred strains to obtain the apparently necessary high degree of heterogeneity in a colony for effective testing of such agents under controlled conditions. They make no mention of representatives of natural populations or their descendents for such tests and yet these would have the requisite heterogeneity.

Studies of laboratory animals can provide basic physiological information obtained under controlled environmental conditions and on uniform genetic backgrounds. However, simulation of natural populations by crossing inbreds is impossible. Inbred strains, as a result of being maintained under more or less controlled unnatural conditions over many generations, have greatly reduced variability as well as certain traits selected for only in laboratory conditions. Therefore there is need for comparison between mice of laboratory populations including inbred strains and natural populations. The house mouse could then serve as a model system in the study of mammalian populations.

The material reported here covers three general areas of information:

(1) the toxicity (as LD_{50}) of 1,1-Bis-(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT), to two inbred strains, as well as some information on the relative susceptibility of mice derived from wild populations.

(2) the effects of p,p'-DDT on four enzyme systems in the mouse.

(3) the oxygen consumption of two inbred strains and some wild house mice.

Inbred strains C3HeP/FeJ and C57BL/10J, obtained from The Jackson Laboratory (Bar Harbor, Maine) were used for the determination of LD_{50}'s. However, for some of the work involving enzymes and oxygen consumption, as well as the matings with wild house mice, animals from the colony at the University of Windsor were used. Although these mice originated as strains C3HeB/FeJ and C57BL/10J from The Jackson Laboratory, the University of Windsor colony has been separated from the main stock for several years, this colony actually deriving from mice obtained via the University of Michigan. Because of the possibility that this derived colony now varies slightly from the mice at The Jackson Laboratory, they are designated only as C3HeB and C57BL .

CHAPTER II

TOXICITY OF p,p'-DDT TO TWO INBRED STRAINS AND SOME WILD HOUSE MICE

In recent years, toxic pollutants have increased in the environment to such a level that they could be expected to exert selective pressures on natural populations of a variety of species. Such pressures may result in an increased resistance to, or tolerance of, specific pollutants. This phenomenon is well documented in the arthropods, where resistance can develop within a short time (Brown, 1968). The mechanisms of resistance are varied, ranging from detoxification of the pesticide to alteration of the target enzyme.

However, attempts to develop resistant strains of vertebrates, specifically the house mouse, Mus musculus, have had mixed results. Ozburn and Morrison (1962) found that the house mouse responded to high concentrations of 1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT) over several generations by developing increased tolerance of it (1.7 fold). Similar work by Guthrie et al. (1971) failed to show the development of such tolerance. Both studies were carried out on laboratory strains which had some variability. The latter attributed their finding to the low levels of genetic heterogeneity in the colony from which their test group of mice was chosen and this low heterogeneity did not permit the selection of a tolerant genotype.

The existence of numerous polymorphic loci has been well established by Petras et al. (1969) and Selander and Yang (1969) for natural populations of Mus. If heterogeneity is as important as suggested by

Guthrie et al. then natural populations could reflect increased tolerance to DDT and similar materials which are now widespread in our environment.

To establish the existence of such increased tolerance, a reliable baseline for comparison must be established. The following describes the development of a baseline which involves the LD_{50}'s of two inbred mouse strains, and a preliminary measurement of the level of tolerance of DDT in natural populations of Mus musculus.

Materials and Methods

1. Source of inbred strains.

The two strains used, C3HeB/FeJ and C57BL/10J, were obtained from The Jackson Laboratory (Bar Harbor, Maine). The mice arrived when approximately five weeks old and were permitted to adjust to their new environment for a week prior to treatment. These strains are known to differ genetically at a number of loci, including those controlling several biochemical variants, and were originally derived from different stocks (Roderick et al., 1971; Staats, 1966). Inbred strains were used because they have been isolated from the natural environment for many generations, and presumably have always been protected from significant exposure to DDT. Two strains were used to permit interstrain comparison. Only one age group (6 weeks ± 3 days) was used in order to minimize possible differences due to factors associated with aging, such as fat accumulation.

2. Mice derived from natural populations.

The mice used to derive information about the level of tolerance of DDT in natural populations were the offspring of crosses between wild males live-trapped from corn cribs near Wheatley, Ontario, and females of inbred strain C3HeB. Any genetically-based resistance present in the males would be inherited by these offspring, although the degree of resistance in the offspring may be intermediate between those of the parents. Ten of these offspring were injected with p,p'-DDT (800 mg/kg) when they reached six weeks of age.

3. Administration of p,p'-DDT and collection of data.

1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT) was obtained in a highly purified form (99%+ purity, Aldrich Chemical Co.). It was dissolved in corn oil (Mazola) for injection intraperitoneally.

Based on preliminary injection studies with doses from 165 to 810 mg/kg, the doses administered were 600,650,700,750, and 800 mg/kg. These were obtained by varying the concentration of p,p'-DDT in corn oil solution. The volume of solution injected varied with the weight of the animal, each mouse receiving 20 ul of solution per gram of body weight. Animals were maintained on a normal diet of Purina Lab Chow and water ad libitum, until death occurred or symptoms of intoxication had disappeared (about two weeks after injection).

4. Methods of analysis of data.

Two different methods were used to calculate LD_{50}:

(a) Probit analysis (as outlined by Fisher and Yates,1957):

This method is based on transforming the proportion of deaths occurring with each dose to a "probit" value which is based on the deviation from a frequency of death of 0.50. Each probit is weighted according to the amount of information which it contributes, the weighting factors being distributed as a normal curve with its maximum value when the frequency of death is 0.50. The method then consists of calculating a weighted regression line of Y on X, where Y is in "probits", and X is the logarithm of the dose given. The LD_{50} is the value of X at a calculated Y of 5.00 (the probit when the frequency of death is 0.50.).

(b) Reed-Muench analysis (as given by Woolf, 1968):

This method is based on the theory that a mouse surviving a high dose would also survive any lower dose. Conversely, any mouse dying at a low dose would also succumb to any higher dose also. Therefore all survivors at a given dose are added to the number of survivors at all lower doses. Similarly, all deaths are added to the number of deaths at all higher doses.. This artificially increases the number of animals tested at any given dose, and the calculated proportion of deaths at each dose is based on these increased numbers. The LD_{50} is calculated by linear interpolation between the two nearest values to 0.50 mortality.

Results and Discussion

The mice of each inbred strain were divided into groups of 10 mice (5 females and 5 males) and each group was given one of the five doses. The mortality results are given is Tables 1 and 2.

The LD_{50}'s based on all the dose groups (600-800 mg/kg) are given for C3HeB/FeJ and C57BL/10J in Table 3. They are, respectively, 733.5 and 662.4 mg/kg, estimated by probit analysis; and 736 and 672 mg/kg, estimated by Reed-Muench analysis. The dose-mortality regression line, estimated by probit analysis, for C3HeB/FeJ, for these doses, is shown graphically in Figure I and the corresponding line for C57BL/10J is shown in Figure II.

The X^2 value obtained for the probit calculated line for C57BL/10J is significant at the 5% level. This may indicate that the data do not fit a straight line and so the line calculated is of dubious reliability (Fisher and Yates, 1957)). Reexamination of the data showed that unexpectedly high mortality occurred when a dose of 600 mg/kg was given. This group of 10 mice was injected at a later date than the initial set of 40 mice (doses 650-800 mg/kg). During this time, the original type of injection syringe and needle were discarded and a different syringe and smaller gauge needles were used. This could have altered the accuracy of the volumes injected. Therefore the LD_{50}'s were recalculated using only the data from the original sets of 40 C57BL/10J (doses 650-800 mg/kg) and 40 C3HeB/FeJ (doses 600-750 mg/kg) mice. This elimination of some data results in very little change in the LD_{50} value for C3HeB/FeJ, however, the LD_{50} value for C57BL/10J becomes higher and the slope of the calculated dose-mortality line is

TABLE 1. Dose-Mortality data for C3HeB/FeJ with pp'-DDT

Dose (mg/ Kg)	# Injected	# Died
600	10	0
650	10	0
700	10	2
750	10	6
800	10	10

TABLE 2. Dose-Mortality data for C57BL/10J with pp'-DDT

Dose (mg/ Kg)	# Injected	# Died
600	10	6
650	10	2
700	10	4
750	10	7
800	10	10

Table 3. Summary of LD_{50}'s calculated for inbred strains C3HeB/FeJ and C57BL/10J

Method of calculation	Strain	Doses (mg/kg)	LD_{50} (mg/kg)	95% limits (mg/kg)
Probit				
	C3HeB/FeJ	600-800	733.5	709-759
	"	600-750	737.7	712-810
	C57BL/10J	600-800	662.4	536-720
	"	650-800	706.2	672-736
Reed-Muench				
	C3HeB/FeJ	600-800	736	711-764
	"	600-750	733	708-759
	C57BL/10J	600-800	672	631-712
	"	650-800	712	680-745

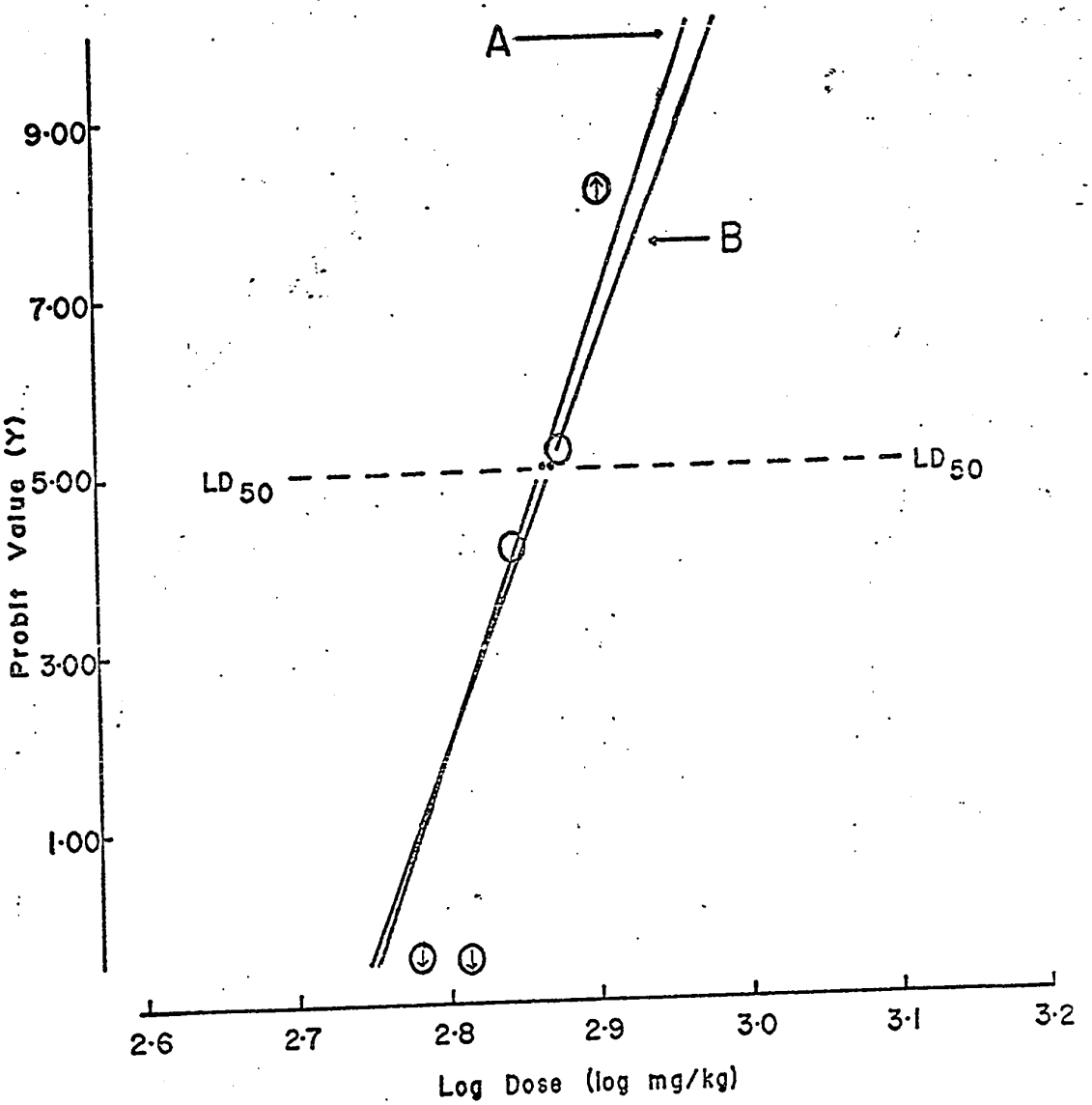


Figure I. Dose-mortality line for C3HeB/FeJ

A = line calculated from data from doses 600-800 mg/kg

B = line calculated from data from doses 600-750 mg/kg

○ = original values calculated from data of Table I.

⊖ = $Y = -\infty$ (all mice survived dose)

⊕ = $Y = +\infty$ (all mice died at this dose)

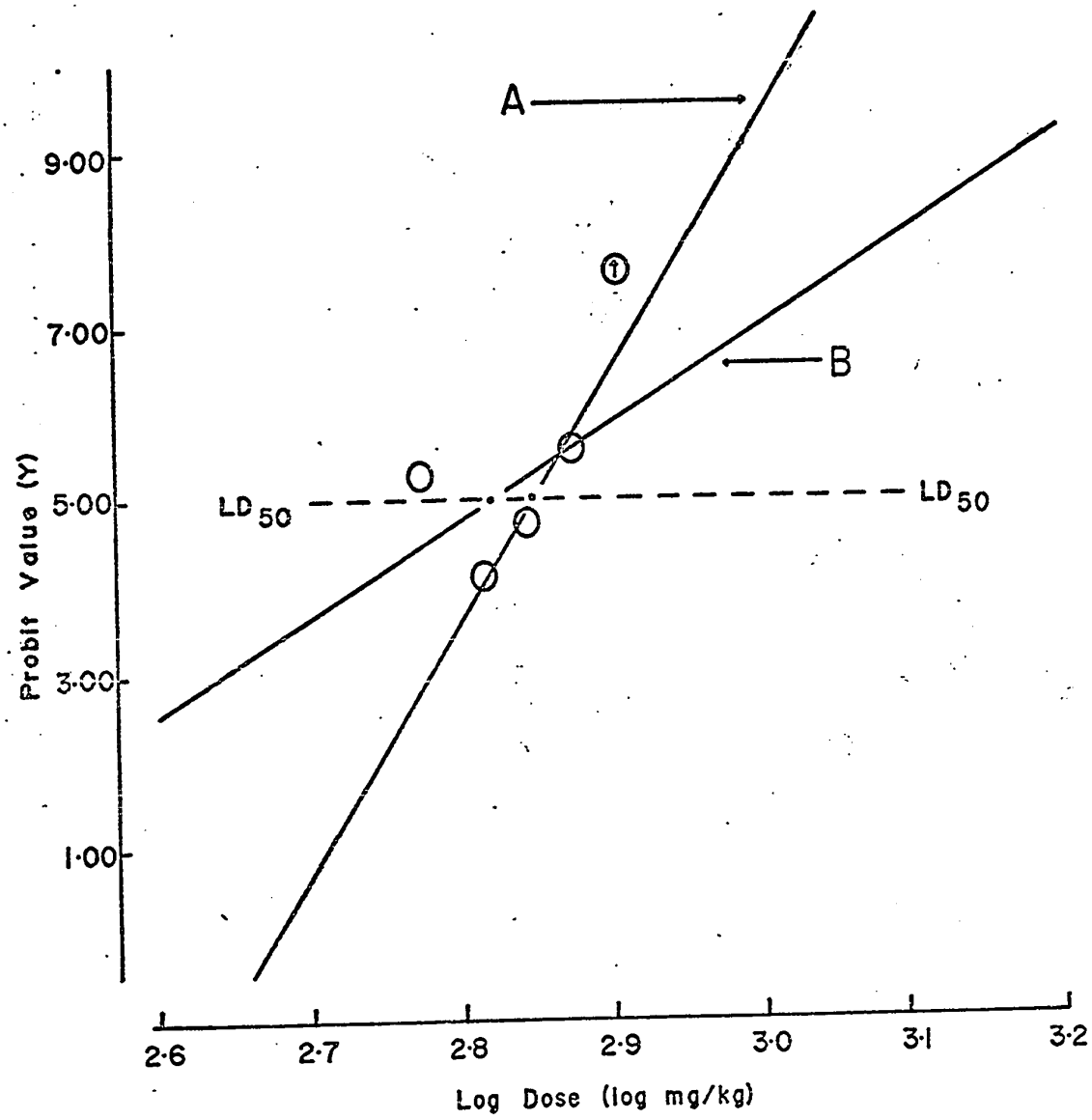


Figure II. Dose-mortality line for C57BL/10J

A = line calculated from data from doses 650-800 mg/kg

B = line calculated from data from doses 600-800 mg/kg

○ = original values calculated from data of Table 2.

⊖ = Y = $-\infty$ (all mice survived dose)

⊕ = Y = $+\infty$ (all mice died at this dose)

steeper. These results are summarized in Table 3 and revised dose-mortality lines are shown for comparison with the originals in Figures I and II. The revised LD_{50} for C3HeB/FeJ is 738 mg/kg by probit analysis, and 733 mg/kg by Reed-Muench analysis. For C57BL/10J, the revised LD_{50} is 706 mg/kg by probit analysis, and 712 mg/kg by Reed-Muench analysis.

The two methods of analysis of the data do not appear to lead to different conclusions. The Reed-Muench analysis is preferable in terms of speed and ease of calculation. The above is consistent with Woolf's statement that the two methods produce similar results with large samples. In this case the sample size is not very large, but Reed-Muench analysis seems to work as well as the more laborious probit analysis.

In the case of probit analysis, 95% "fiducial limits" have been calculated to aid in the comparison of LD_{50}'s, however, quantitative comparison is not given by this method. With Reed-Muench analysis, LD_{50}'s may be compared by the "C test" (Woolf,1968). Using this method, the two inbred strains differ significantly in their mean LD_{50}'s (Table 4). Since the 95% fiducial limits of probit analysis given in Table 3 agree quite well with the 95% confidence limits of Reed-Muench analysis, it appears that the validity of the Reed-Muench analysis and the C test is corroborated. The degree of increased tolerance of C3HeB/FeJ is not great, being only 1.03 times that of C57BL/10J. While it is statistically significant, the increase in tolerance is negligible for practical reasons. Ozburn and Morrison(1964) calculated the LD_{50} in their control colony to vary from 500 mg/kg to 570 mg/kg over several

Table 3. Comparisons of mean LD_{50}'s of C3HeB/FeJ and C57BL/10J by "C test"

Group	C value	p
All mice injected	164	<.001
Restricted to same day injection	84	<.001

generations without consistent trends. Furthermore, Guthrie et al (1971) found that the LD_{50} of their test colony could vary from about 570 mg/kg to as high as 730 mg/kg without apparent differences in inherited resistance developing. The difference between C3HeB/FeJ and C57BL/10J is very small when compared to the difference in tolerance (1.7 fold) reported by Ozburn and Morrison (1962) for their test and control colonies. The 1.03 fold difference found in this work could easily be accounted for by experimental error in determining the LD_{50}. Such error could be due to variation in the method or in the nongenetic background of the inbred strains.

Considering the amount of experimental variation that can occur, the LD_{50}'s of the two strains reported here, those reported by Ozburn and Morrison for their control strain, and the values given by Guthrie et al can be regarded as similar. This places the expected LD_{50} of laboratory and inbred strains in the range of 500-750 mg/kg.

If the variation in the calculated LD_{50} is this great for relatively uniform laboratory mice, then considering the degree of heterogeneity in mice from natural populations (Selander and Yang,1969), the degree of increased tolerance present in such a population would have to be of considerable magnitude to be detected. If the work of Webb and Horsfall (1967) with the pine mouse (Pitymys pinetorum) is a reliable indicator, then resistance in heavily exposed natural populations may reach over 10 fold (in their case with respect to endrin) relative to unexposed populations.

Preliminary work with offspring of matings between wild male house mice and the inbred strain C3HeB does not, however, indicate that

such resistance is the rule in natural populations of Mus. F_1 mice were used in order to control the background and age of the mice injected, and therefore eliminate some variation associated with age differences and previous exposure to DDT. Because of the small number of mice available (10), only one relatively high dose was used rather than trying to estimate and LD_{50}. This method is more likely to detect resistance should only a small proportion of the tested animals be resistant (Mouchet, 1968).

Of the 10 mice injected with 800 mg/kg p,p'-DDT, none survived. While this represents offspring from only three wild males, it suggests that resistance, if present, is probably not the rule but perhaps the exception in the populations of house mice examined. Further work is needed to provide a definite estimate of the level of DDT tolerance in wild house mice, and to determine the validity of Guthrie et al. on variability and tolerance to DDT.

The availability of inbred strains of Mus allows techniques to be used which were unavailable to Webb and Horsfall for Pitymys pinetorum. Since they worked with live-trapped wild Pitymys, it is impossible to determine the source of the resistance which they found. While it is possible that the resistance was genetically based, the question arises of whether this could be a case of induced resistance whereby Pitymys responds to exposure to a pesticide such as endrin with an increased ability to tolerate it, possibly through the induction of degradation systems for endrin.

However, since inbred strains of Mus are available, the technique of using F_1 mice of matings of wild males and inbred females can be used to control factors such as age and pesticide exposure. This allows

investigation of genetically based and induced resistance to be done separately.

Summary

(1) The estimated mean LD_{50} for C57BL/10J, derived from probit analysis, is 706 mg/kg, for intraperitoneal injection of p,p'-DDT, with 95% fiducial limits of 672 and 736 mg/kg. Reed-Muench analysis of the same data for C57BL/10J provides and estimated LD_{50} of 712 mg/kg, with 95% confidence limits of 680 and 745 mg/kg.

(2) The estimated mean LD_{50} for C3HeB/FeJ, derived via probit analysis, is 738 mg/kg, for intraperitoneal injection of p,p'-DDT, with 95% fiducial limits of 712 and 810 mg/kg. Reed-Muench analysis of the same data gives an estimated LD_{50} of 733 mg/kg, with 95% confidence limits of 708 and 759 mg/kg.

(3) The C test based on Reed-Muench analysis indicates that C57BL/10J and C3HeB/FeJ differ significantly in the mean LD_{50} values ($p < .001$); however, for practical use the difference between the LD_{50}'s is negligible. The values are similar to those reported for other colonies (Ozburn and Morrison,1964; Guthrie et al ,1971) in a general range of 500 to 750 mg/kg.

(4) Preliminary evidence does not indicate a greater tolerance of p,p'-DDT by wild house mice to be the rule in natural populations.

CHAPTER III

EFFECTS OF p,p'-DDT ON FOUR ENZYME SYSTEMS

DDT (1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane) is one of innum rable pollutants in the environment. It is certainly one of the most widespread, being found not only in areas where is is known to have been applied, but also in areas greatly removed, such as the arctic region (Cade et al, 1971). Because of its molecular stability and progressive concentration along food chains, it is one of the most serious of pollutants. Of immediate concern are such effects as the "raptor-pesticide syndrome" which has led to the population declines of several raptor species (Cade et al, 1971; Bitman et al, 1970; Peakall, 1970).

The long-term genetic effects of DDT are not yet clear. Wallace (1970) reported that mice from a population previously exposed to high levels of DDT contained an abnormally high proportion of mutants. Petras et al, (1969) have suggested that some of the polymorphisms found in natural populations may be responses to an alteration of the environment by, for example, the introduction of synthetic organic compounds such as pesticides. That is, pollutants such as DDT may be placing selective pressures on the genotypes of natural populations. Cory et al, (1971) maintain that this is the case in natural populations of Drosophila in California, where changes in the relative frequencies of a number of chromosomal inversions correlate with the introduction of DDT in a number of areas.

Although there is evidence from in vitro work that the primary target of p,p'-DDT in vertebrates is the enzyme Na^+,K^+-activated,Mg^{++}-

dependent adenosine triphosphatase (Matsumura and Patil,1969; Janicki and Kintner,1971), the evidence provided by Cory et al. of selection at the chromosomal level suggests that DDT may have a broad spectrum of effects.

In this study, four enzyme systems were examined to determine whether any of these enzymes may be among "target systems" of DDT.

Materials and Methods

1. Treatment of animals and preparation of homogenate extracts.

Mice used were strains C3HeB/FeJ and C57BL/10J purchased from The Jackson Laboratory, or were the equivalent strains C3HeB and C57BL from the University of Windsor colony. They were injected intraperitoneally with p,p'-DDT dissolved in corn oil at doses of 600 to 800 mg/kg.

Kidneys and livers were taken from mice:

(a) found in convulsions and killed by cervical dislocation;

(b) found dead ;

(c) apparently recovered from symptoms of intoxication and killed by cervical dislocation;

(d) killed by cervical dislocation-no injection (controls).

Kidney samples were prepared by homogenization of both kidneys with 0.5 ml. of 1% (v/v) Triton X-100 in distilled water. The glass pestle was motor driven and the conical tube immersed in ice water during the homogenization. Liver samples were prepared similarly except that 1.0 ml

of 1% Triton X-100 was used. In both cases this represents approximately a 1:1 dilution of tissue with liquid.

Homogenates were frozen and thawed slowly three times. Following the third thawing, they were centrifuged twice at 27,000 xG (in a Sorvall RC2-B) for one hour each time. Electrophoresis and histochemical staining of enzyme activity was carried out according to methods used by Hutton (see Hutton and Roderick,1970).

2. Biochemical systems studied.

(a) Glucose-6-phosphate dehydrogenase (G6PD):

A key enzyme in carbohydrate metabolism, this enzyme helps control glucose flow into the glycolytic pathway. The NADPH produced in the reaction is utilized in reductive biosynthesis, especially the synthesis of fatty acids (Lehninger, 1970). In the house mouse, the structure is a dimer controlled by one locus (Gpd-1) with two alleles: $Gpd\text{-}1^a$ in C57BL and $Gpd\text{-}1^b$ in C3HeB (Ruddle et al, 1968).

(b) Cytoplasmic $NADP^+$-malate dehydrogenase (MDH):

The oxidation of malate to pyruvate + carbon dioxide by this "malic enzyme" is also a source of NADPH for fatty acid synthesis (Lehninger, 1970). Malic enzyme also catalyzes the reverse reaction and may also function as an anaplerotic mechanism to supply malate to the TCA cycle when needed (Wada et al, 1968). In the house mouse it has a tetrameric structure controlled by one locus (Mod-1) with two alleles: $Mod\text{-}1^a$ in C3HeB and $Mod\text{-}1^b$ in C57BL (Baker and Mintz,1969).

(c) Cytoplasmic $NADP^+$-isocitrate dehydrogenase (IDH):

This enzyme is involved in the "shuttle" of intra-mitochondrial reducing power to form extra-mitochondrial NADPH (Lehninger,1970). In the house mouse this enzyme is apparently a dimer controlled by one locus (Id-1) with two alleles: $Id\text{-}1^a$ in both C57BL and C3HeB and $Id\text{-}1^b$ in several other strains. (Henderson,1965).

(d) Lactate dehydrogenase (LDH):

Five isozymes exist for this enzyme in mammals. The structure is a tetramer with two subunits; i.e. two loci control the five isozymes. The kinetic properties of the various isozymes differ with respect to affinity for pyruvate and the rate of oxidation of NADH (Lehninger,1970).

Lactate dehydrogenase and G6PD were chosen for study as a result of reports by Sova (1966) for LDH and Tinsley (1964) for G6PD of inhibition of these enzymes by p,p'-DDT and because electrophoretic techniques are available for them. G6PD, IDH, and MDH supply NADPH for biosynthesis. With the exception of LDH, these enzymes are all polymorphic in natural populations of the house mouse.

3. In vitro tests for malic enzyme inhibition by p,p'-DDT.

The in vitro effects of p,p'-DDT on malic enzyme were examined in samples obtained from non-injected mice. The samples were homogenized with solutions of p,p'-DDT dissolved in a concentration of 40 or 180 ppm in 1% Triton X-100 plus 5% dimethylformamide (DMF) in distilled water

as follows:

(a) For the determination of the effects of the solvents used as well as the p,p'-DDT, 2 C57BL and 2 C3HeB mice were killed , their livers removed, and each liver was minced with a scalpel and divided into three equal parts. The liver was homogenized as follows:

i) one third of a liver with 0.3 ml Triton X-100;

ii) one third of the same liver with 0.3 ml Triton X-100 plus 5% DMF;

iii) one third of the same liver with 0.3 ml Triton X-100 plus 5% DMF plus p,p'-DDT(40 ppm). This gave an approximate concentration of 20 ppm DDT in the homogenate.

(b) For the determination of the effects of p,p'-DDT , eight C3HeB mice were killed, their livers were removed, and were homogenized as follows:

i) one liver with 1 ml. 1% Triton X-100 (solvent control 1);

ii) one liver with 1 ml. 1% Triton X-100 plus 5% DMF (solvent control 2);

iii) six livers, each with 1 ml. 1% Triton X-100 plus 5% DMF plus p,p'-DDT (180 ppm). This gave an approximate concentration of 90 ppm DDT in the homogenate.

Results and Discussion

The basic zymograms and deviations of the enzymes are summarized in Table 5. Included are the number of animals examined in each category

Table 5. Enzyme activity in kidney extracts of mice injected with p,p'-DDT

Enzyme	Strain	No Injection	Convulsive	Dead	Recovered
LDH					
	C3HeB	2 +++++[a]	4 +++++	4 +++++	4 +++++
	C57BL	6 +++++	14 +++++	13 +++++	13 +++++
G6PD					
	C3HeB	2 +	2 +	3 + 1 split[b]	3 +
	C57BL	5 +	12 +	8 +	8 +
IDH					
	C3HeB	2 +	2 +	4 +	4 +
	C57BL	4 +	8 +	9 +	10 +
MDH					
	C3HeB	5 +	2 + 1 -[c]	1 + 3 -	4 +
	C57BL	5 +	0 + 7 -	3 + 6 -	5 + 3 -

[a] +Indicates a normal pattern. For LDH each of the 5 bands was considered individually; hence 5 +'s for a complete normal pattern. See Figure III.

[b] split; indicates an extra band not usually found in the pattern. See Figure III.

[c] -; indicates loss of enzyme activity; i.e. a very light band on starch gel. See Figure III.

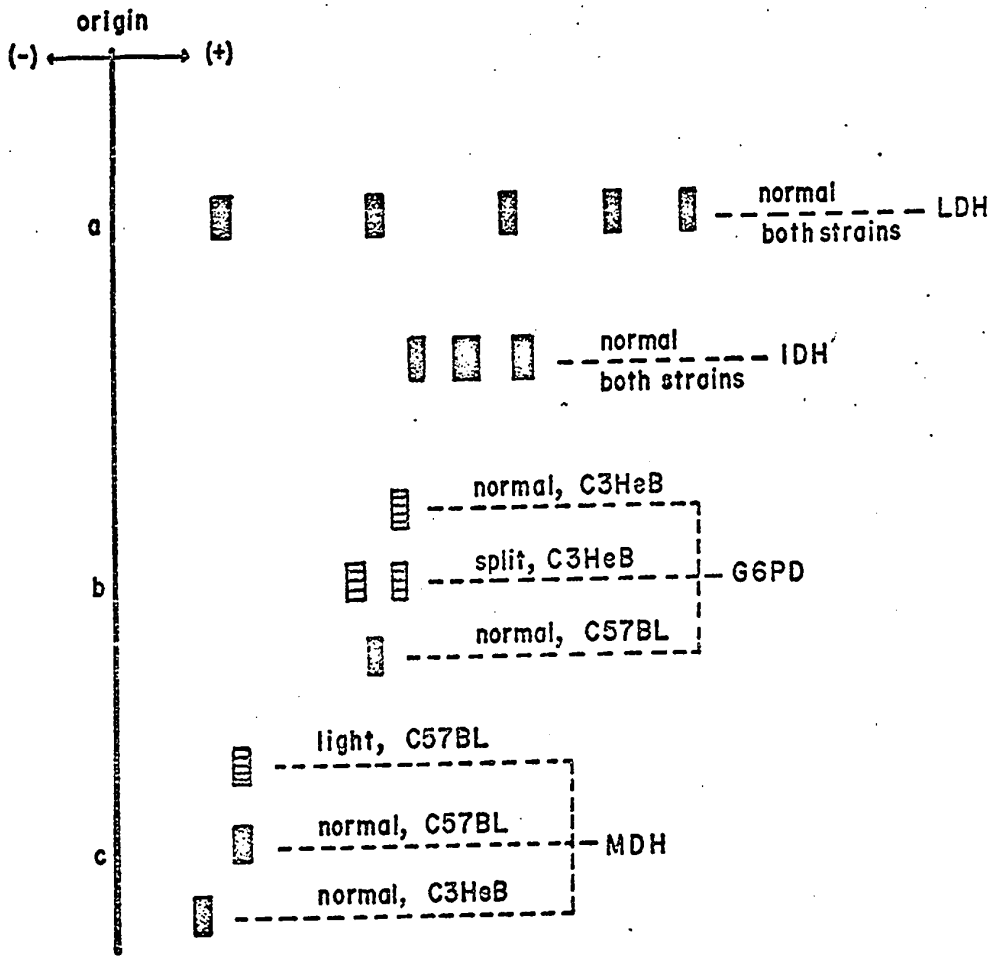


Figure III. Patterns of enzyme activity referred to in Table 5.

(control, survivors, convulsive when killed, or dead) and the type of pattern found in these animals. Figure III is a diagram of the various patterns referred to in Table 5.

Sova (1966) reported that rabbit muscle lactate dehydrogenase activity is completely inhibited by in vitro preincubation with DDT at a concentration of 10^{-4} M (about 35 ppm). However he did not attempt to detect inhibition of LDH from animals treated with DDT. In this study, no loss of activity was seen on the electrophoretic patterns of LDH from mouse kidneys even when lethal doses were used.

Pocker et al, (1971) found that bovine carbonic anhydrase, although reported by Keller (see Pocker et al, 1971) to be inhibited by p,p'-DDT in vitro, was not inhibited by this compound, but was coprecipitated with DDT when the solubility limits of that compound in 5% DMF were exceeded. The enzyme could be recovered by redissolving the DDT precipitate. Considering the method used by Sova (pre-incubation) his results may be spurious, reflecting not inhibition but entrapment of LDH with precipitating DDT.

Tinsley (1964) reported that in vitro treatment of rat liver homogenates with p,p'-DDT in concentrations of 10^{-5} M inhibited G6PD activity. Furthermore, rats fed diets containing p,p'-DDT had reduced G6PD activity in liver homogenates. Inhibition was not seen on starch gel with homogenates from mice. This may reflect the presence of insensitive G6PD in the mouse, although it is quite probable that the percentage inhibition would have to be high to be detected with this method. The single case of a split pattern occurred in a mouse which had been found dead and this may be an artefact caused by some decomposition o occurring before the liver was removed and frozen.

Both inbred strains used had the same IDH pattern, that is, they are homozygous for the same allele (Id-1^a). In neither did this enzyme appear to be affected by p,p'-DDT *in vivo*. Figure III shows that the IDH pattern consisted of a multiple band pattern rather than the single band reported for this allele by Henderson (1965). Since this was characteristic of the controls as well as the DDT-injected mice, this is presumably a storage effect occurring during the thawing-freezing treatment.

Cytoplasmic $NADP^+$-malate dehydrogenase (malic enzyme) presents the only case of greatly reduced or lost activity after administration of p,p'-DDT. Animals found in convulsions often had very low or negligable malic enzyme activity in kidney extracts, and in liver extracts as well. However, since none of the livers which were homogenized with p,p'-DDT gave evidence of malic enzyme inhibition, direct interaction does not seem to be the answer. This material is summarized in Table 6.

The probable cause of loss of malic enzyme activity in mice found in convulsions or dead due to DDT intoxication is induced repression or inhibition of NADP-MDH as a result of fasting. Lehninger (1970) states that this enzyme is not active in liver preparations of fasting rats, and Wada *et al*, (1968) demonstrated that livers of fasting rats contain a heat-stable component which inhibits cytoplasmic NADP-MDH in liver preparations of normal rats. Since the convulsions due to DDT intoxication can last several hours, the mice are probably in a fasting state and unable to eat. The time after onset of convulsions to the discovery

Table 6. *In vitro* effect of p,p'-DDT on malic enzyme

Concentration	Strain		Triton X-100	+ DMF	+ DDT
20 ppm[a]	C3HeB	A	light	light	light
	"	B	dark	dark	dark
	C57BL	A	dark	dark	dark
	"	B	dark	dark	dark
90 ppm	C3HeB		1[b] dark	1 dark	6 dark

[a]The 20 ppm data represent 4 livers--2 each from strains C3HeB (animals A and B) and C57BL (animals A and B). Each liver was divided into three equal parts, then each part treated differently.

[b] Number of animals used.

of the condition and killing the mouse could affect the degree of inhibition, hence mice killed soon after convulsions started could have normal malic enzyme activity. This would explain the normal enzyme activity found in two of the three convulsive C3HeB mice in Table 5.

In addition to possible inhibitory effects of DDT, there is the well-established phenomenon of liver enzyme induction by DDT (Hoffman et al, 1970; Schwark and Ecobichon, 1968; Morello, 1965; Peakall, 1970). No evidence of increased enzyme activity was noted on the starch gels, but then this could be obscured by the semi-quantitative nature of starch gel electrophoresis. Although superior to most other forms of electrophoresis for a qualitative demonstration of isozymes, starch gel is a distinct liability when quantitative work, such as is needed for studies of enzyme inhibition or induction, is required. In order to work quantitatively, the gels must be made transparent by soaking in a glycerol solution, after which isozyme activities can be measured by transmittance densitometry. The alternative method is to freeze the gel and elute samples for assay (Gordon, 1969).

Furthermore, starch gel and other forms of electrophoresis are limited as to the enzymes for which methods are available. For example, Na^{+},K^{+}-activated,Mg^{++}-dependent adenosine triphosphatase is not active once removed from the cell membrane (Lehninger, 1970), hence the free protein could not be detected by an enzyme catalyzed reaction after electrophoresis. This is unfortunate since Matsumura and Patil (1969) suggest that two forms of this enzyme exist in rat brain neurons, only one of which is DDT-sensitive. Although the two proteins could probably be separated by electrophoresis there would be great difficulty in detecting them.

Summary

(1) No apparent inhibition of enzyme activity by p,p'-DDT was seen in dead or convulsive animals with respect to:

a) lactate dehydrogenase (LDH)

b) glucose-6-phosphate dehydrogenase (G6PD)

c) cytoplasmic $NADP^+$-isocitrate dehydrogenase (IDH)

Thus although Sova (1966) reported inhibition of LDH *in vitro* with p,p'-DDT, and Tinsley (1964) found inhibition of liver G6PD in rats fed p,p'-DDT, there is no demonstrable loss of activity of these enzymes, or of cytoplasmic IDH, in mice injected with p,p'-DDT, even though some mice were killed or severely intoxicated by the doses of DDT given.

(2) Cytoplasmic $NADP^+$-malate dehydrogenase is often inhibited or absent in livers and kidneys of mice found in convulsions or dead after injection of p,p'-DDT. It may also be decreased in animals recently recovered from symptoms of DDT intoxication.

(3) Since liver tissue mixed with p,p'-DDT *in vitro* by homogenization does not lose malic enzyme activity, it is doubtful that loss of activity *in vivo* is due to direct inhibition of the enzyme by p,p'-DDT.

(4) It is known that fasting rats show inhibition of malic enzyme activity and that a thermostable inhibitory factor exists in the liver supernatant of starved rats (Wada *et al*, 1968). It appears that enzyme activity decreased in mice in convulsions due to fasting conditions in the mice.

CHAPTER IV

COMPARISON OF METABOLIC RATES OF TWO INBRED STRAINS AND WILD HOUSE MICE

In 1962, Ozburn and Morrison reported the development of a strain of laboratory mice, Mus musculus, tolerant to DDT (1,1-Bis-(p-chlorophenyl)-2,2,2-trichloroethane). Subsequently they described their protocol as well as some of the strain's reproduction and growth characteristics (Ozburn and Morrison, 1964). They found no differences in litter size and sex ratio between the "tolerant" colony and the control colony. The tolerant mice did, however, appear to be slightly heavier at four weeks of age.

When examined for metabolic rate response to DDT injection, Ozburn and Morrison (1965) found that both groups respond to a sublethal injection with a rise in oxygen consumption, however, the tolerant mice show a lesser increase in oxygen consumption than the control mice. Furthermore, at 25 days old, mice from the tolerant colony had a higher oxygen consumption than the controls even though no treatment had been given.

Ozburn and Morrison felt that the difference in oxygen consumption between the young mice of the two colonies confirmed the presence of a physiological difference between them, although they were derived from the same stock.

To determine whether a detectable physiological difference exists between inbred strains C3HeB and C57BL, the resting metabolic rates, as measured by oxygen consumption were determined and examined for

significant differences. In addition, the metabolic rate of wild house mice (Mus musculus) was determined and compared with that of the inbred strains.

Materials and Methods

1. Source of inbred strains.

The mice used were females of two inbred strains. These were either obtained from The Jackson Laboratory (strains C3HeB/FeJ and C57BL/10J) or were the equivalent strains (C3HeB and C57BL) from the University of Windsor colony. In the discussion only the designations C3HeB and C57BL are used to refer to the strains. Animals were six to eight weeks old when tested.

2. Source of wild house mice.

The wild female mice used were either laboratory-born animals from a female pregnant when trapped, or were litters found when trapping mice in corn cribs and fostered in the laboratory by a female from one of the laboratory strains. All the wild mice were seven to nine weeks old (estimated) when tested.

3. Apparatus and measurements.

The metabolism apparatus used is only slightly modified from the one used by Ozburn and Morrison (1965). The apparatus is shown in Figure IV.

Figure IV. Schematic diagram of oxygen metabolism apparatus (one chamber shown) (adapted from Ozburn and Morrison,1965)

a = 15 cm. dessicator

b = wire cage to contain mouse

c = petri dish

d = indicator soda lime

e = thermometer, ^{o}C

f = stoppered Y-tube

g_1 and g_2 = dissicator-buret connecting tubing

h = 250 ml. dispensing-type buret

i = test tube water leveller

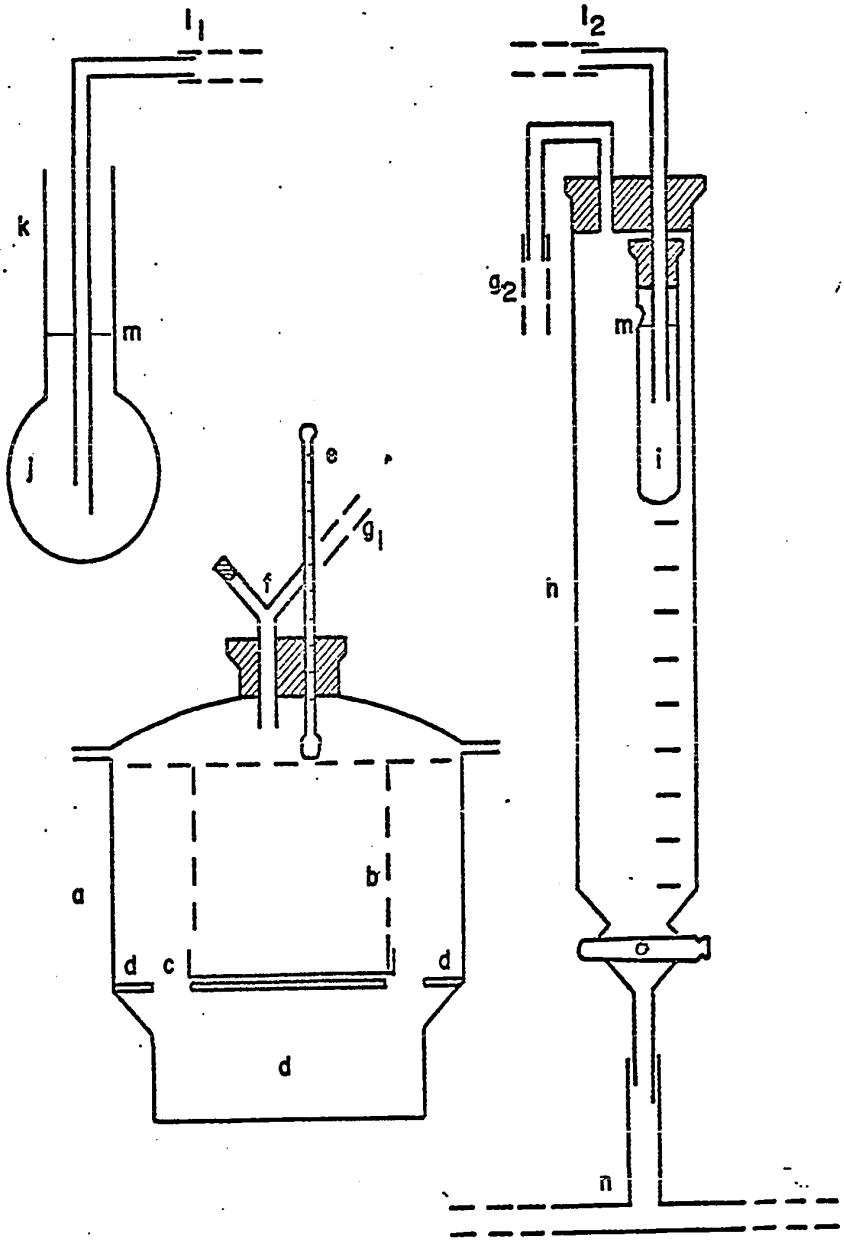
j = glass water supply tube (shown in cross-section at one riser tube)

k = glass riser tube

l_1 and l_2 = water siphon tubing

m = water level of system

n = drain system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Female mice of the same strain were placed in five of the six chambers for each set of measurements. The sixth chamber was used as a control to measure the effect of increased pressure in the system on the water level in the buret, and to permit appropriate adjustment of the readings. Decreases in pressure within the system could not be detected and therefore if these changes occurred, the volume readings would be slight underestimates. There is, however, no reason to suspect that this occurred.

For the first set of measurements, mice were placed in the test chambers and the water level in each buret was adjusted to the 250 ml. mark. The system was then sealed and left for 80-100 minutes. At the end of this time the volume of water in the buret was recorded. The change in volume of water was assumed to be equivalent to the volume of oxygen consumed by the mouse. This volume was then corrected to STP and adjusted for weight. The oxygen consumption was thus calculated by the formula:

$$O_2 \text{ consumption} = \frac{(V) \cdot (P_s) \cdot (T_v)}{(w) \cdot (P_v) \cdot (T_s) \cdot (t)}$$

where:
- V = change in water volume(ul)
- P_s = standard pressure (1013.25 mb)
- T_v = ambient temperature (^oK)
- w = weight of the mouse (g)
- P_v = ambient pressure (mb)
- T_s = standard temperature (273 ^oK)
- t = elapsed time (minutes)

Units: μl/g/minute

When the change in volume of water in the buret had been recorded for set 1, the water level was again adjusted to the 250 ml. mark. The

system was again sealed for 80-100 minutes and a second reading of increased water volume was taken. This constituted the second set of measurements. Oxygen consumption was calculated as for set 1. The strains and wild mice were compared by Student's t test of averages for unpaired readings. Variances within test sets were compared by F test of the ratio of variances of sets (Simpson et al, 1960)

Results and Discussion

Two sets of metabolic rate measurements are given in Table 7 for the inbred strains. The first set was based on 30 C3HeB and 20 C57BL. A second set of measurements made immediately after the first (as described in Materials and Methods) involved 25 C3HeB and 15 C57BL mice. In both strains, the oxygen consumption was significantly lower during the second set of measurements. This was unexpected since subjective observation of the mice during some preliminary tests in the apparatus indicated that changes in activity levels were random over the duration of the test, and as a result the second set of measurements had been made on most animals. The intent was to provide two sets of observations on each mouse and to average the two to determine oxygen consumption. However, it became clear as the data were compiled that there is a trend for oxygen consumption to decrease between the two sets, therefore each set is presented separately.

This significantly higher oxygen consumption in set 1 posed the question of reliability of the two sets of measurements. An analysis of the data showed that the results from set 1 had a greater variance

Table 7. Oxygen consumption of female C3HeB and C57BL mice and some female wild house mice

Set of measurements	Strain	No. of mice	O_2 consumption (ul/g/min)	Standard error
1				
	C3HeB	30	66.2	17.9
	C57BL	20	64.2	15.8
	Wild	10	86.6	14.2
2				
	C3HeB	25	52.8	12.3
	C57BL	15	54.4	11.8
	Wild	10	70.5	12.4

($ml/min/kg^{.75}$) not convertible to those used here.

In the final portion of this study, the average oxygen consumption of 10 female wild mice was determined. The values for both sets of measurements are given in Table 7. The pattern of decreased oxygen consumption from set 1 to set 2 is consistent with the observations for the inbred strains, so the wild mice are reacting similarly in the apparatus. However, the average oxygen consumption of the wild mice is significantly higher in both sets of measurements than either of the inbred strains. This varies from the findings of both Pearson and Morrison who reported data which give average values of 58.3 and 65.0 ul/g/min for wild house mice over a 24-hour period.

Several explanations can be suggested for the different values:

(1) Pearson's and Morrison's values are based on a 24-hour period of testing whereas the values reported here are based on a considerably shorter period. The testing time had no effect when laboratory strains were involved, but may have an effect when wild mice or their descendents are concerned.

(2) Their data are based on a very few mice; Pearson's value on two mice and Morrison's on only one. Furthermore the ages are not specified; the value for an older animal may be lower than that for a young adult.

(3) Finally, there is also the possibility that the natural populations in this area differ from the ones from which those authors obtained their mice.

In conclusion, although Pearson's and Morrison's methods differ from the one used in this study, the similarity of the results for inbred strains and for the laboratory mice used by Pearson and by Morrison

suggests this is not the cause of the difference in values for wild mice. There is no apparent reason to reject the higher value of 86.6 ul/g/min obtained here as being characteristic for female wild house mice in the populations sampled. Therefore there may be a basic physiological difference between mice of wild populations and the inbred and laboratory strains.

Since Ozburn and Morrison (1965) reported that a higher oxygen consumption in young mice was correlated with an increased tolerance to p,p'-DDT, does a similar correlation exist in wild mice? Unfortunately in this case, the female wild mice tested for oxygen consumption were not young enough (6 weeks ± 3 days) to determine their response to p,p'-DDT at 800 mg/kg, and the F_1 mice (wild x C3HeB) mice injected with p,p'-DDT had not been tested for oxygen consumption. Further work remains to be done in this regard.

Summary

(1) For C57BL mice (females 6-8 weeks old) the average oxygen consumption, when measured without prolonged adaptation to the apparatus (set 1) was 64.2 ul/g/min (standard error=17.9 ul/g/min). For the same mice, tested after 80-100 minutes adaptation to the apparatus (set 2), the average oxygen consumption was 54.4 ul/g/min (S.E.= 11.8 ul/g/min).

(2) For C3HeB mice (females 6-8 weeks old) in set 1, the average oxygen consumption was 66.2 ul/g/min (S.E.=15.8 ul/g/min). For the same mice in set 2, the mean oxygen consumption was 52.8 ul/g/min (S.E.=12.3 ul/g/min).

(3) The oxygen consumption of both strains in set 1 is significantly higher than in set 2. This may reflect less activity by the mice after confinement in the apparatus for some time, and a change toward basal metabolism after a few hours of food deprivation.

(4) The oxygen consumption of the two strains C3HeB and C57BL do not differ significantly when measured in either set 1 or set 2.

(5) The oxygen consumption values obtained for the inbred strains agrees closely with the values previously reported by Pearson (1947) and Morrison (1947) for laboratory mice.

(6) The oxygen consumption of wild house mice (10 females 7-9 weeks old) determined in this study was 86.6 ul/g/min when measured in set 1 and 70.5 ul/g/min in set 2. Both of these values are higher than previously reported values (Pearson,1947; Morrison, 1947). Although several possible explanations were proposed, the data do not favour any particular one.

CHAPTER V

GENERAL DISCUSSION

Considerable research has been done using the laboratory mouse, Mus musculus, as the experimental subject. Its physiology, development, structure and response to many agents have been studied. Probably more is known about its genetic makeup than that of any other mammal except man. However this form of the mouse is very different from the original wild house mouse from which it has descended. While the development of the laboratory and inbred strains have been an enormous aid in allowing researchers to focus on specific characteristics to separate genetic effects from experimentally induced effects, these mice may not react in a given set of circumstances in the same way that animals from natural populations would. Thus when it is suggested by Palmes and Pup (1970) that laboratory or inbred strains or dihybrid crosses between inbred strains can be used to develop laboratory populations as the test subjects to evaluate the effects of environmental agents, only a portion of the obtainable information becomes available.

Animals kept under laboratory conditions have been, deliberately or not, selected for a variety of traits such as docility, high reproductive rate, larger size, etc. and at the same time numerical bottlenecks occur. The end result is not only elimination of mutants because of selection, but also the loss of alleles because of genetic drift, so that inbred strains or even a 4 or 8 strain cross may no longer carry the variability found in natural populations. For example, Ruddle and Roderick (1968) noted that only one of 15 inbred strains

lack of suitable genetic variation in the original experimental animals. The work of Selander and Yang (1969) and Petras et al, (1969) leave no doubt that natural populations of the house mouse are highly heterogeneous. Therefore it is probable that natural populations of the house mouse in habitats where they are directly exposed to p,p'-DDT develop levels of tolerance greater than populations not directly exposed. If this is so, then whether the mechanism and degree of tolerance is identical to that found by Ozburn and Morrison in laboratory mice remains to be explored.

The determination of the LD_{50}'s of two inbred strains, C57BL/10J and C3HeB/FeJ, has led to the conclusion that laboratory and inbred strains thus far examined have LD_{50}'s in the range of 500-750 mg/kg, the value being affected by variation in the method and nongenetic background of the test animals. Preliminary data on the F_1 offspring of crosses between wild male mice and inbred strain C3HeB indicate that natural populations in habitats not directly exposed to p,p'-DDT have no greater tolerance than laboratory mice, however, the LD_{50} has not been calculated for statistical comparison. Since the mice derived from natural populations appear to have roughly the same tolerance to p,p'-DDT as the two inbred strains reported here, then these strains would be suitable to use in further studies of natural populations to provide a baseline for comparison.

Since the basis of any resistance is at the biochemical level, this is where the effects of p,p'-DDT should be sought. Evidence presented by Matsumura and Patil (1969) and Janicki and Kintner (1971) indicates that Na^+,K^+,-activated, Mg^{++}-dependent adenosine triphosphatase (NaKA) inhibition in vitro correlates with the toxicity of p,p'-DDT to the organism from which the adenosine triphosphatase was obtained. In addition, other enzymes have also been reported as being inhibited

by p,p'-DDT. Yap et al (1971) report that muscle Mg^{++}-dependent adenosine triphosphatase is even more sensitive than NaKA. Hiltibran (1971) found that p,p'-DDT inhibits electron flow from succinic acid to the cytochrome chain as well. Tinsley (1964) found that liver glucose-6-phosphate dehydrogenase activity from rats fed a diet containing 100 ppm p,p'-DDT was markedly reduced. Furthermore, since p,p'-DDT also reduced the enzyme's activity in vitro at a concentration as low as 7 ppm, he concluded that the inhibition was due to direct interaction. Sova (1966) had reported that rabbit muscle lactate dehydrogenase was inhibited completely by in vitro pre-incubation with p p'-DDT (10^{-4}M). In this study no inhibition due to direct interaction with p,p'-DDT was found with respect to murine lactate dehydrogenase, glucose-6-phosphate dehydrogenase, cytoplasmic $NADP^{+}$-isocitrate dehydrogenase, or cytoplasmic $NADP^{+}$-malate dehydrogenase (malic enzyme) examined by histochemical staining on starch gels.

In the present study, loss of malic enzyme activity had occurred in most mice which were killed when found in convulsions. There was no inhibitions however, in liver homogenates with p,p'-DDT in concentrations of 20 or 90 ppm. The reason for the loss of enzyme activity in convulsing mice may be repression of malic enzyme associated with a fasting state. Wada et al, (1968)reported that fasting rats had depressed malic enzyme activity.

Many enzymes may be affected by DDT, the effects on a number of which may result in loss of vital activity and thus be lethal. Despite the evidence provided by Matsumura and Patil (1969) and Janicki and Kintner, (1971) that NaKA inhibition could account for the toxicity of p,p'-DDT, which of many enzymes is the primary target of this compound has yet to be determined.

A number of physiological differences may exist between laboratory mice and individuals from natural populations. Although females of the two inbred strains C3HeB and C57BL exhibit very similar oxygen consumption, female wild house mice of similar age, laboratory-reared, have a significantly higher oxygen consumption. While the inbred strains have an oxygen consumption very close to values reported for laboratory mice by Pearson (1947) and Morrison (1947), the value obtained here for wild house mice conflicts with their values for wild mice which were close to the values obtained for laboratory mice. It will require measurements on mice from other populations under conditions of age and sex similar to those used here to determine whether Pearson's and Morrison's values are low due to factors such as age of their mice, or small sample size, or the values obtained here are high because of the different method of measurement used, or variation between this population and those studied by Morrison and Pearson. Should such differences between laboratory and wild mice be the rule rather than the exception it would be of interest to determine whether this affects the response to pesticides such as p,p'-DDT. Unfortunately the F_1 (wild x C3HeB) mice were not tested for oxygen consumption prior to injection with DDT, nor were the female wild house mice which were tested for oxygen consumption young enough (6 weeks ± 3 days) to determine their response to p,p'-DDT at 800 mg/kg for comparison with the F_1's.

In conclusion, it is suggested that the use of wild house mice or their descendents form an important part of the study of mammalian systems. While inbred strains provide opportunities to work with biological problems on a uniform genetic background, wild mice provide genetic

heterogeneity which may serve as the basis for such characteristics as the development of resistance to pesticides, adaptations to environmental changes, and mechanisms of evolution. The study of the house mouse as a model mammalian population would be better done through the combined use of members of natural populations of Mus musculus, their descendents, and the laboratory strains.

CHAPTER VI

GENERAL SUMMARY

1. LD_{50}'s of 1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT) for two inbred strains of the house mouse, Mus musculus, have been determined and compared. The LD_{50} for C3HeB/FeJ calculated by probit analysis was found to be 738 mg/kg with 95% fiducial limits of 712 and 810 mg/kg. Calculated by Reed-Muench analysis the same data provide andestimated LD_{50} of 733 mg/kg with 95% confidence limits of 708 and 759 mg/kg.

2. The $LD_{50}$for C57BL/10J obtained by probit analysis was found to be 706 mg/kg, with 95% fiducial limits of 672 and 736 mg/kg. Calculated by Reed-Muench analysis, the same data provide an estimated mean LD_{50} lf 712 mg/kg with 95% confidence limits of 680 and 745 mg/kg.

2. The two methods of analysis used gave similar results with the sample size chosen. Because of the relative ease and speed of calculation Reed-Muench analysis is preferable to the more laborious probit method.

3. A slight difference in tolerance between C3HeB/FeJ and C57BL/10J was significant at the 1% level as determined by "C test" using the mean LD_{50}'s and variances calculated by Reed-Muench analysis. However, since the diffference is slight (733 versus 712 mg/kg) and larger variation has been reported elsewhere for different generations of the same laboratory strain without apparent genetic basis, the difference reported here may be attributed to variations in experimental procedure or in the nongenetic background of the mice rather than significant tolerance differences. In general the laboratory and inbred strains of

mice examined to date have LD_{50}'s in the range of 500-750 mg/kg.

4. Preliminary evidence based on a single dose of 800 mg/kg given to ten F_1 mice from a cross of wild house mice with inbred strain C3HeB indicates that natural populations in habitats not directly exposed to p,p'-DDT have a tolerance level no greater than that of the inbred strains reported here.

5. p,p'-DDT administered in vivo to inbred strains C3HeB and C57BL of Mus musculus does not produce significant effects on the pattern or intensity of staining on starch gels of the enzymes lactate dehydrogenase, glucose-6-phosphate dehydrogenase, or cytoplasmic $NADP^+$-isocitrate dehydrogenase. Cytoplasmic $NADP^+$-malate dehydrogenase (malic enzyme) activity is depressed in kidney extracts and liver extracts of most convulsive mice as well as those from some mice which have died of DDT intoxication. The apparent explanation for this occurrence is inhibition of malic enzyme induced by a fasting state.

6. Female mice of inbred strains C57BL and C3HeB were tested for oxygen consumption using a method similar to that of Ozburn and Morrison (1965). When mice were tested without allowing a prolonged period of adaptation to the apparatus, C57BL had an average oxygen consumption of 64.2 ul/g/min over a 90-minute period. Under the same conditions, C3HeB mice had an average oxygen consumption of 66.2 ul/g/min.

7. In measurements made after approximately 90 minutes of adaptation to the apparatus, the oxygen consumption for C57BL dropped to an average of 55.4 ul/g/min over a further 90 minute period. Under the same conditions, the oxygen consumption of C3HeB also dropped, to a value of 52.8 ul/g/min. In both strains this decreased value is signifi-

cantly different from the one obtained in measurements made without allowing a prolonged period of adaptation to the apparatus. The lower value may represent decreasing oxygen consumption as metabolism is changing from a resting to a basal state.

8. Because of the correspondence of the values obtained without a period of adjustment to the apparatus to values obtained by Pearson (1947) and Morrison (1947) as averages over 24-hour measurements of laboratory mice, this method seems suitable for estimating "resting" or average metabolic rates by oxygen consumption.

9. The oxygen consumption of female wild house mice determined by measurements made without allowing a lengthy adaptation period in the apparatus had an average value of 86.6 ul/g/min. This value is significantly higher than the value for either inbred strain tested. This is not in accord with the reports of Pearson (1947) and Morrison (1947) who found that wild house mice have an oxygen consumption similar to that of laboratory mice. The differences observed may be the result of any of several factors (age differences, sample size, population variability), however the results do not favour any particular explanation.

10. It is suggested that natural populations of Mus musculus or their descendents are better suited for use in the investigation of biological problems dealing with genotype-environment interaction than are the laboratory strains.

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