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Natural populations of Mus musculus variability in response to warfarin in blood clotting times and reproductive patterns.

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NATURAL POPULATIONS OF MUS MUSCULUS: VARIABILITY IN RESPONSE TO WARFARIN, IN BLOOD CLOTTING TIMES, AND REPRODUCTIVE PATTERNS

by

David Geoffrey Morgan

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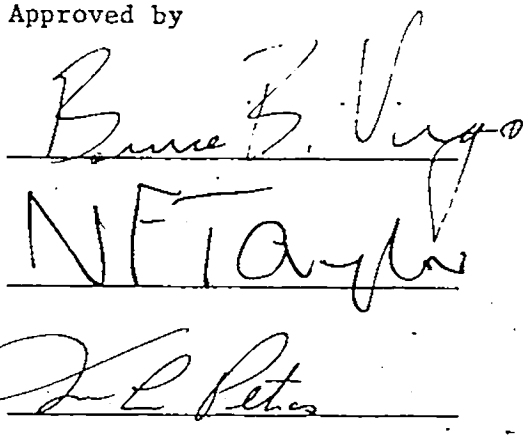

ABSTRACT

NATURAL POPULATIONS OF MUS MUSCULUS: VARIABILITY IN RESPONSE TO WARFARIN, BLOOD CLOTTING TIMES, AND REPRODUCTIVE PATTERNS

by

David Geoffrey Morgan

Resistance to warfarin is a trait involving a specific physiological response to a pharmacological factor, and as such differs from morphological and biochemical systems typically examined in mammalian populations. As a result of this difference, study of warfarin resistance gives an additional dimension to studies of genetic variability in natural populations.

Variability in response to the blood anti-coagulant warfarin was examined in 112 wild mice from 20 populations in south-western Ontario and 8 from New York State. As controls, 27 known resistant warfarin outcross animals [C57BL/6J x PBI (MAFF)] were utilized. The testing procedure involved giving the mice food which contained 0.025 percent warfarin for 21 days. In the natural populations from Ontario, resistance was found in 26.6% of animals tested. Based on a two allele with dominance mode of inheritance, the frequency of the allele responsible for resistance (War?) was 0.14 and 0.08 in the Ontario and New York samples respectively, as compared to 0.18 reported for British populations (Rowe and Redfern, 1964 and 1965). No association was detected between the resistance trait and two

polymorphic loci (Hbb and Gpi-1) known to be linked (War?).

Using the Quick One-Stage Prothrombin Time Test (Quick, 1966) blood clotting was examined in inbred, wild, known warfarin resistant and susceptible as well as warfarin treated Mus musculus. Differences in prothrombin times among different individuals should reflect concentration differences in blood clotting factors, expecially factors VII and/or X, both of which are directly measured by Quick's Method and are affected by warfarin. No significant differences in prothrombin times were detected among the test groups, even after modifications of the testing procedure were carried out. Insensitivity of the testing method could prevent detection of actual differences in prothrombin times which may exist.

Finally, studies on the variability in reproductive patterns of Mus musculus in corn crib populations in south-western Ontario were undertaken. Pre and post-implantation mortality estimates were obtained using corpora lutea counts and uterus examinations at autopsy. Using starch gel electrophoresis, correlation was sought between embryonic mortality and maternal genotype at six loci. Results of this study were compared with those of Batten and Berry (1967) from natural populations of Mus musculus in Britain. Embryonic mortality estimates for the house mouse populations examined ranged from 1.3% to 37.7% ($\bar{X}$ = 18.2 $\pm$ 12.7) for pre-implantation loss, 0.0% to 18.3% ($\bar{X}$ = 8.1 $\pm$ 6.2) for post-implantation loss, and 13.8% to 42.6% ($\bar{X}$ = 24.0 $\pm$ 10.0) for total prenatal loss. Overall prenatal mortality was lower in the Canadian populations than in the British samples (31.1% to 36.2%).

Other results of this study include, mean litter size among the populations (5.97 ± 0.24), a significant positive correlation between litter size and maternal weight, no statistically significant differences between the two uterine horns for such parameters as live and resorbed embryo numbers, and little variability of embryonic mortality among the study populations in terms of time of year the sampling was done, and geographic location of the population. There appeared to be no pattern of association between pregnancy parameters and specific genotypes, as determined by electrophoretic typing of a number of protein systems.

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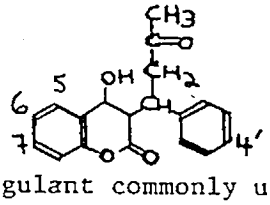
CHAPTER I

INTRODUCTION

Natural populations of Mus musculus provide a good system for examining genetic variability in mammals. Most often, biochemical and morphological systems are examined using such techniques as electrophoresis or physical measurement. Electrophoretic studies, for example, have shown substantial amounts of genetic variability at such loci as Hbb and Gpi-1 (Hoeg, 1979).

Variability in response to the blood anti-coagulant warfarin is a trait which permits studies distinct from electrophoretic and morphological investigation of polymorphisms in natural populations. Determining whether an animal lives or dies after exposure to warfarin measures genetic variability in a different manner from the situation where coat color or protein molecule charge differences may serve as indicators. Variability in response to warfarin may be examined using a toxicity test.

Warfarin, [3-(α-acetonylbenzyl) - 4 - hydroxycoumarin] is an



indirect blood anticoagulant commonly utilized as a rodenticide. Reports in the literature have described populations of mice and rats, resistant to the compound (Greaves et al., 1976a). Failure to control rodent infestations by the use of warfarin can, of course, have serious repercussions in terms of damage to stored food-stuffs such as

grain.

Mode of Inheritance of Warfarin Resistance

Reports of inter-individual differences in response and resistance to warfarin have appeared in the literature for at least four species, Mus musculus, Rattus norvegicus, R. rattus and man. The mode of inheritance of resistance is quite well understood in each of these species.

Rowe and Redfern (1965) first proposed that resistance to warfarin in Mus musculus might be a heritable trait. Wallace (1974a, 1974b, 1975) briefly described the selection of warfarin resistant mice from animals trapped in a warfarin treated area in Cambridge, England. Linkage studies were also carried out using outcross matings to a warfarin susceptible marker stock. These test results along with comparison studies using mice from three wild-caught British mouse populations (Wallace and MacSwiney,1976; MacSwiney and Wallace, 1978) established that in these British populations, warfarin resistance is controlled by the single major gene War and penetrance is affected by sex, and one or more modifiers. Wallace and MacSwiney (1976) state that the locus War is located on chromosome 7, close to the locus frizzy. In the same paper, dominance of the gene was shown to be both sex-limited and modifiable in males by age and the residual genotype. Wallace and MacSwiney (1976) also proposed that a "cream" mutant, or the gene responsible for the coat colour extreme chinchilla (c^e) had persisted and spread throughout the wild

population at Cambridge due to linkage with the War gene.

Resistance to warfarin in wild populations of Rattus norvegicus has been observed in Scotland, Denmark, Wales, Holland and the United States (Greaves and Ayres, 1976b). In the Welsh rat population warfarin resistance has been shown to be due to a single dominant autosomal gene at the Rw locus (Rw^2). The Rw locus maps in Linkage group 1 and is linked to the c and p loci (Greaves and Ayres, 1969; O'Reilly et al., 1968). The position of the Rw gene is located in a homologous position to the War gene in the mouse (MacSwiney and Wallace, 1978). In a wild R. norvegicus population from Scotland, a resistance gene Rw^3 was identified (Greaves and Ayres, 1976b). This gene was found to be allelic with the Welsh gene Rw^2 but unlike the Rw^2 gene which has complete penetrance, the Rw^3 gene was shown to have imcomplete penetrance controlled by modifiers. This point is interesting for several reasons. Not only is the gene for warfarin resistance in some rat species in a homologous position on the chromosome to the mouse resistance gene, but it also appears to be regulated in the same fashion. It is possible, that the gene for warfarin resistance is very old, having originated before these rodent species diverged from a common ancestor.

Work done with R. rattus from Liverpool populations (Greaves et al., 1976) showed that resistance was heritable, but in a manner different from the clear-cut single gene types fround in R. norvegicus.

Inter-individual differences in response to warfarin exist and are heritable in man also, however, the mode of inheritance is not as well

understood as for the mouse and the rat. Preliminary study indicates that as is seen in rodents, differences in warfarin response in man are heritable in a dominant fashion. O'Reilly et al. (1964, 1967) describe humans resistant to warfarin treatment. One male required 145 mg of warfarin per day to achieve anticoagulative response. This value is about 20 times the normal mean daily dose of 6.8 ± 2.8 mg. Pedigree analysis indicated that warfarin resistance was inherited as an autosomal dominant Mendelian trait.

Solomon (1968) described a patient who was susceptible to warfarin. A weekly dose of only 2.5 to 5.0 mg was all that was required to produce a spinal cord hematoma severe enough to result in paraplegia.

Inter-individual differences in response to warfarin can therefore be shown to exist in a number of different species, and there appears to be a number of similarities between species in the mode of inheritance of this genetically variable trait.

Mechanism of Resistance

While warfarin resistance is known to exist and be heritable, the actual mechanism of resistance is not clearly understood. A very large volume of literature exists, dealing with potential sources of inter-individual differences at various sites along the metabolic pathway of warfarin. The basic metabolic pathway consists of warfarin being absorbed from the gut and more than 97 percent loosely bound to plasma albumin (Goodman and Gilman, 1975; Yacobi et al., 1976a and b; O'Reilly and Kowitz, 1967; Oester et al., 1976). Free drug is

metabolised in the liver by cleavage of the heterocyclic ring of warfarin (Lush and Arnold, 1975; Lewis and Trager, 1970). Since warfarin is largely bound to plasma proteins, little glomerular filtration of the unchanged drug takes place, and due to the drug's lipid solubility, any free unchanged drug is reabsorbed from the renal tubules. Metabolites of warfarin do not exhibit these properties and are eliminated (O'Reilly et al., 1962).

Warfarin binding to plasma albumin has been proposed as a possible site for warfarin resistance to occur and many aspects of this phenomenon have been investigated. Although serum albumin levels, total protein concentrations and concentration of free warfarin do not vary in rats and man (Yacobi et al., 1976a), different concentrations of total warfarin are required to elicit a defined anticoagulant effect (Yacobi and Levy, 1975a). Small inter-individual variations in warfarin-albumin association constants and in the number of warfarin binding sites per albumin molecule (0.9 to 2.1) were shown to be influenced by genetic factors using monozygotic twin studies (Wilding et al., 1977c; Yacobi et al., 1976b). Conformational differences in albumin, endogenous displacing agents or endogenous binding inhibitors and reduced warfarin binding albumin variants have been mechanisms reported to explain differences in response to warfarin (Yacobi and Levy, 1975b; Sjoholm et al., 1976; Wilding et al., 1977a). A large number of factors influence warfarin-albumin interactions and these also have been related to inter-individual differences in response to the drug; many drugs increase or decrease

warfarin-albumin binding (Goodman and Gilman, 1975), age (neonates and the elderly) can cause decreased binding of warfarin (Hayes et al., 1975; Ehrnebo et al., 1975), many disease states reduce the binding capacity for warfarin (Bachmann and Shapiro, 1977; Yacobi and Levy, 1977), endogenous substances such as free fatty acids can increase warfarin-albumin binding at low concentrations, but decrease binding efficiency at higher concentrations (Nilsen et al., 1977; Chakrabarti et al., 1976; Wilding et al., 1977b). Both environmental and genetic factors could therefore influence inter-individual differences in warfarin-albumin binding and, as a result be expressed as differences in resistance to the drug.

Much research has also been based on the hypothesis that the mechanism of warfarin resistance may be due to variation in metabolism of the drug. Different species, for example, have been shown to differ for such parameters as warfarin tolerance and plasma half-life of the drug (Nagashima and Levy, 1969; Goodman and Gilman, 1975).

In inbred mice such as DBA/2J and C57BL/6J, genetic variation exists in coumarin hydroxylase activity, a liver microsomal cytochrome P450 enzyme actually comprised of products from two closely linked genes, and responsible for the 7-hydroxylation step in the degradation of warfarin (Wood and Conney, 1974; Lush and Arnold, 1975; Lush and Andrews, 1978). It was shown however, that no correlation exists between hydroxylating ability and warfarin resistance. Townsend et al. (1975) concluded for rats, that the rate of metabolism of warfarin was not the rate limiting step as far as the lethal effects of warfarin

were concerned.

Several authors claim that because warfarin appears to act by inhibiting vitamin K oxide reductase, and therefore blocks the biosynthesis of blood clotting factors II, VII, IX and X, an alteration in the reductase, so that it is less efficient and less sensitive to warfarin inhibition, could be the mechanism for warfarin resistance (Caldwell et al., 1974; Bell and Caldwell, 1973; Zimmermann and Matschiner, 1974; Greaves and Ayres, 1976a; Whitlon et al., 1978). If this hypothesis is correct, resistant animals would have higher vitamin K requirements than susceptible animals. Differences in vitamin K requirements necessary to counteract the effects of warfarin have been shown for several strains of inbred rats (Greaves and Ayres, 1973).

There are opponents to this hypothesis also (Stenflo and Suttie, 1977) and there is therefore no clear, single explanation which covers the mechanism for warfarin resistance.

Warfarin, a Teratogen

Warfarin has been shown to be embryo toxic in such species as cattle, dogs, rabbits, and mice. No increase in the frequency of malformations has been observed, however, newborn animals with hemorrhages have been found (McCallion et al., 1971; Wright, 1951; Kraus et al., 1949; Hirsh et al., 1970; Kronick et al., 1974).

In humans, warfarin given early in the first trimester of pregnancy often results in an embryopathy with such distinctive

features as nasal hypoplasia, blindness and retardation (Burdi and Barr, 1976; Laros, 1970; DiSaia, 1966).

This study utilized a toxicity test to examine response to warfarin in natural Mus populations in North America.

Variability in Blood Clotting Times

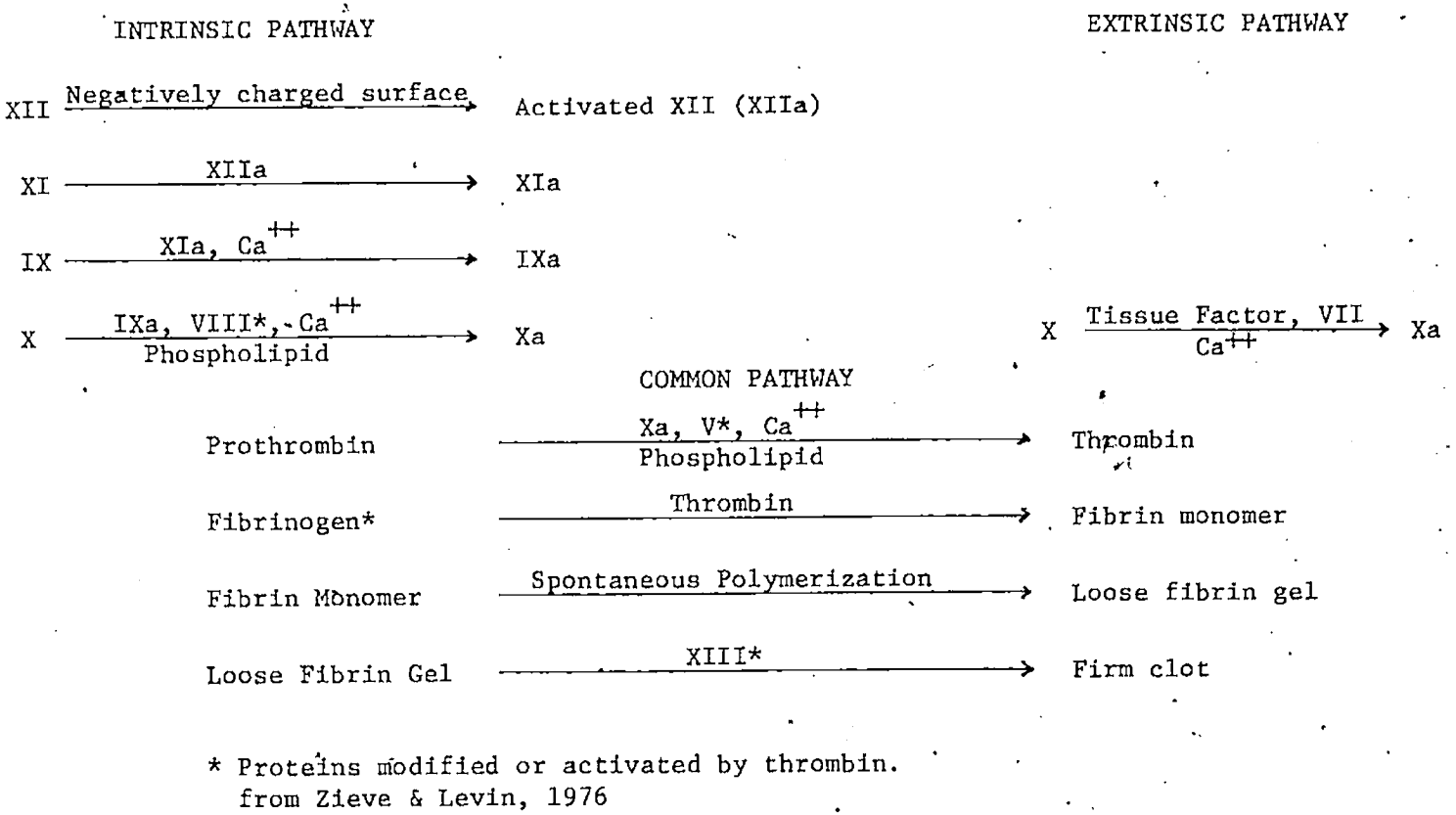
Blood clotting times as determined by prothrombin time measurements were established to a) determine if variability in clotting times existed among animals and b) determine if any variability found could be associated with the warfarin resistance trait. Prothrombin time was therefore examined, to see if this would be a suitable phenotype for studying variability, and one that could be readily applied to warfarin resistance survey work.

Blood coagulation results from a series of cascading reactions which enable fluid plasma to solidify at blood vessel damage sites to prevent undue loss of blood. The complex coagulation system is composed of 13 coagulation proteins, most of which circulate as inactive pro-enzymes. Non-protein substances such as phospholipids and divalent cations are also required at several points in the system (Zieve and Levin, 1976).

Figure 1 summarizes the steps in blood coagulation. Two sequences, known as the intrinsic and extrinsic pathways have been identified, each with separate initiation conditions.

The Quick One-Step Prothrombin Time Test measures or examines both the intrinsic and the extrinsic clotting pathways. A drawback

Figure 1: Pathways of blood coagulation



* Proteins modified or activated by thrombin.
from Zieve & Levin, 1976

with this test however, is its relative lack of sensitivity. Since the various proteins that participate in coagulation as substrates are usually present in excess, some differences in the concentration of the protein or clotting factors (as much as 20 percent for factor IX) may go undetected. It is partly for this reason, that ranges of 11-15 seconds for prothrombin times are accepted as normal in humans. These ranges may also incorporate variability in clotting time which may have a physiological and/or a genetic basis. In spite of its poor resolution, the Quick One-Stage Prothrombin Time Test is of value in monitoring the effects of warfarin administration on blood coagulation. As described above, warfarin inhibits the synthesis of vitamin K dependent coagulation proteins II, VII, IX and X. Concentration differences in vitamin K dependent clotting factors VII and X may be detected with the Prothrombin time test.

Pre- and Post-Implantation Mortality

Pre- and post-implantation mortality in natural populations of Mus musculus was originally examined for a study, not performed, on the teratological effects of warfarin. These data do however, provide information on embryonic mortility and its variability in the populations examined. Mortality estimates were for populations within the same geographic region as populations used for toxicity testing.

CHAPTER II

VARIABILITY IN RESPONSE TO WARFARIN IN NATURAL POPULATIONS OF THE HOUSE MOUSE, MUS MUSCULUS

As described in the Introduction, natural populations of Mus musculus from Ontario exhibit substantial amounts of genetic variability when a variety of loci are examined using starch gel electrophoresis (Hoeg, 1979). Genetic variability for the gene War, responsible for warfarin resistance, has been shown to exist in British Mus musculus populations (MacSwiney and Wallace, 1978). Because resistance to warfarin is a trait, involving a specific physiological response to a pharmacological factor present in the environment, and as such differs from physical entities such as morphological and biochemical systems typically examined in mammalian populations, it gives an additional dimension to studies of genetic variability in natural populations.

The questions posed in this study include: (1) Is there variability at the War locus in North American Mus populations?; (2) How does this compare with other loci known to be polymorphic?; and (3) Is there any association between two polymorphic loci (Hbb - β chain of hemoglobin and Gpi-1-glucose phosphate isomerase) known to be linked to War and the resistance phenotype?; (4) If resistance to warfarin exists in North American Mus, how do allelic frequencies for the trait compare with those in Britain? Finally, a discussion of additional studies required is also presented.

Rowe and Redfern (1964, 1965) have described a toxicity test

procedure suitable for detecting warfarin resistant mice which they used on wild Mus musculus from corn-ricks in England. The testing procedure involved giving the mice food which contained 0.025 percent warfarin for 21 days.

In the present study, wild mice were collected from populations in south-western Ontario and from the State of New York. The wild mice were compared to known warfarin resistant C57BL/6J outcross PBI and MAFF warfarin resistant mice from England.

Material and Methods

Source of Animals

Wild mice trapped from 20 corn cribs located in Essex and Kent counties in south-western Ontario were utilized in this study. To reduce the possibility of age effects on expression of resistance all animals utilized in the study had been classified as young adults at time of capture during the summer of 1977. None of the females utilized were pregnant at the time of the study.

Wild mice from 8 trapping sites near Stoneybrook, New York, were obtained from A. Miller-Baker. All animals utilized were adults.

Warfarin resistant animals [PBI($\underline{c^e c^e}$), MAFF($\underline{c^e c^e}$)] to be utilized as controls were obtained from England and were outcrossed to C57BL/6J mice to provide numbers of animals adequate for toxicity testing. PBI mice ($\underline{c^e c^e}$) which originated at the Plant Breeding Institute, Trumpington, Cambridge, were obtained from M. E. Wallace,

Cambridge University. MAFF mice ($\underline{c^e c^e}$) were obtained from J. H. Greaves, MAFF laboratory, Hook Rise South, Surbiton, Surrey.

All animals were housed in stainless steel cages at temperatures between 20-24°C and with 12 hours of light per day. Animals were fed Purina Laboratory Chow and given water ad libidium.

Toxicity Testing Procedure

At least two weeks before a test, each animal was isolated in a cage; the week before the start of testing, paper towel sheet bedding was substituted for wood-chip bedding and a diet of powdered laboratory chow, corn oil and molasses was substituted for laboratory chow pellets. Food was presented in a small ceramic food-pot with a capacity of approximately 20 grams of loosely packed powder.

The method employed in all toxicity tests was to offer each mouse excess amounts of feed mixture containing 0.025 percent warfarin for a 21 day period.

In preparing the feed for toxicity testing, warfarin was mixed thoroughly with powdered laboratory chow to give a 0.5 percent master-mix. The master-mix was prepared in 200 gram batches by mixing in a Hobart mixer for several hours. One part of master-mix was then added to 19 parts of a blended feed base, consisting of powdered laboratory chow, molasses and corn oil (17:2:2) and this was again mixed thoroughly for several hours. The warfarin containing mixture was then divided into individual 10 gram units and stored in small plastic bags. Warfarin was obtained from Sigma Chemical Co., St. Louis, Missouri

(lot #56C-0071) and crystaline sodium warfarin was obtained from Endo Laboratories, Garden City, New York (lot #77-043). The amount of warfarin-feed mixture given to each animal was recorded, and spilled mixture was recovered not less frequently than every other day. Animals were weighed at least once a week during the test. The time of death, weight, and external signs of hemorrhage were recorded, and an autopsy to discern signs of internal hemorrhage was performed. Mice still alive at the conclusion of the test period were placed on a diet of a warfarin free feed mixture for one week and then were returned to metal cages with wood-chip bedding and laboratory chow pellets.

Electrophoresis

Animals were bled from the orbital sinus, into a five percent sodium citrate mammalian physiological saline solution (0.8 percent sodium chloride). Blood was processed for starch gel electrophoresis according to the procedure described by Biddle and Petras (1967). The electrophoretic procedure for examining the phenotypes controlled by the Hbb, Gpi-1 and Alb-1 loci is described by Harris and Hopkinson (1977) and Petras et al. (1969).

Statistical analyses were done on a Monroe 1860 calculator.

Results

Toxicity Testing

Table 1 summarizes mean and standard deviation values for animals' weights and toxicity test parameters for the three test groups. The Ontario test group is sub-divided by trapping regions in Essex and Kent counties. More detailed toxicity test data is presented in Table 34 (Appendix A).

Both sexes of mice were utilized for the tests. Weights of the animals, as determined by the mean weight throughout the test period, ranged from 12.0 grams to 31.1 grams (Table 34).

The amount of warfarin-feed mixture eaten per mouse ranged between 2.0 grams and 99.6 grams for the 21 day test periods. This amount is to some extent determined by the length of survival of the test animals. The amount of bait consumed per day per animal ranged from 0.3 grams to 4.7 grams. The total warfarin dose per animal ranged between 28.4 mg/kg and 1250.0 mg/kg of body weight. The first animal to die after the commencement of the test, did so on day 4, and 46 of the 134 mice tested survived the complete 21 day test period (Table 34).

Electrophoretic studies also summarized in Table 34 (Appendix A) revealed the presence of mice with all three possible genotypes represented at each of the Hbb and Gpi-1 loci but only one genotype ($\underline{Alb\text{-}1}^{a}/\underline{Alb\text{-}1}^{a}$) at the Alb-1 locus.

Table 1: Mean weight (± S.D.) of animals and mean results (± S.D.) of toxicity testing with warfarin for the total test group and the warfarin resistant subgroup

Region		Number of Animals	Mean Weight of Animals	Total Food Consumed (gm.)	Food Consumed Per Day (gm.)	Mean Warfarin Dose (mg/kg.)	Mean Day of Death
Ontario	Total	94	17.7 ± 3.2	37.8 ± 24.7	2.3 ± 0.9	525.6 ± 327.0	13.2 ± 5.3
	Resistant	23	18.2 ± 2.0	69.9 ± 11.4	3.3 ± 0.5	968.1 ± 128.7	---
Harrow	Total	18	18.4 ± 2.8	38.4 ± 30.5	2.3 ± 1.2	526.1 ± 421.1	11.7 ± 3.0
	Resistant	6	18.3 ± 3.1	77.7 ± 11.2	3.6 ± 0.5	1073.6 ± 134.5	---
Central Essex	Total	31	18.6 ± 4.2	42.6 ± 22.9	2.6 ± 0.7	581.2 ± 294.7	14.5 ± 6.5
	Resistant	7	18.8 ± 4.1	68.1 ± 13.6	3.2 ± 0.6	921.7 ± 132.0	---
Stoney Point	Total	7	17.1 ± 1.9	41.0 ± 22.1	2.4 ± 0.6	588.7 ± 310.8	14.4 ± 7.0
	Resistant	2	16.5 ± 1.2	62.1 ± 4.2	3.0 ± 0.2	932.6 ± 3.6	---
Dover Township	Total	34	16.9 ± 2.4	33.5 ± 23.8	2.1 ± 0.9	473.8 ± 313.8	12.7 ± 4.8
	Resistant	8	18.1 ± 1.8	67.5 ± 8.8	3.2 ± 0.4	934.0 ± 104.5	---
Ontario	Control	3	19.0 ± 4.8	54.4 ± 4.2	2.6 ± 0.2	---	---
New York	Total	13	21.9 ± 4.1	26.2 ± 15.6	1.8 ± 0.6	301.7 ± 171.8	12.9 ± 5.0
	Resistant	2	26.6 ± 1.4	53.6 ± 17.0	2.6 ± 0.8	509.2 ± 187.2	---
New York	Control	2	22.6 ± 0.3	52.0 ± 6.2	2.5 ± 0.3	---	---
PBI/MAFF	Total	27	22.0 ± 3.2	47.2 ± 18.4	2.4 ± 0.7	544.3 ± 206.8	14.5 ± 5.0
	Resistant	19	22.1 ± 3.3	56.1 ± 12.8	2.7 ± 0.6	639.7 ± 133.6	---

Means and Standard Deviations of Toxicity Test Results

Table 1 shows that in both the Ontario and New York samples, animals that survived the toxicity test (mean weight = 18.2 ± 2.9 grams and 26.6 ± 1.4 grams respectively) were not much heavier than animals which died (mean weight = 17.7 ± 3.2 grams and 21.9 ± 4.1 grams respectively). There was little weight difference between survivors and those animals which died within the known resistant PBI/MAFF control sample.

Mean amounts of warfarin-feed mixture consumed during the toxicity tests ranged between 26.2 grams and 54.4 grams among the three samples, but this value is related to the number of animals which survived. In all cases, animals fed control feed-mixture consumed more than animals presented warfarin-feed mixture. With the exception of mice from New York, the mean daily feed consumption was fairly constant, ranging between 2.3 and 2.6 grams. The mean amount eaten per day by warfarin resistant animals i.e. those from all three test groups which survived the test period, ranged between 2.6 and 3.3 grams and was higher than the mean values for the test groups as a whole, and for the control animals.

The mean warfarin dose ranged from 301.7 mg/kg to 544.3 mg/kg for the three test samples as a whole and between 509.2 mg/kg and 968.1 mg/kg for resistant animals.

Mean day of death for each of the three toxicity test samples ranged from 12.9 to 14.5 days with a mean of 13.5 days. Table 2 presents the same data for each test group but sub-divided for sex.

Table 2: Mean weight of animals and mean results of toxicity testing with warfarin for the total test group and the warfarin resistant group

Sample		Animal Weight	Weight (Resistant Animals)	Total Food Consumed	Food Consumed Per Day	Total Food Consumed (WR)	Food Consumed Per Day/WR	Mean Warfarin Dose (mg/kg.)	Mean Warfarin Dose/WR (mg/kg.)	Mean Day of Death
Ontario Wild	M	18.2 (±3.3)* 74**	18.9 (±2.9) 17	39.2 (±24.9) 74	2.4 (±0.9) 74	73.0 (±11.4) 17	3.4 (±0.6) 17	533.8 (±319.7) 74	972.1 (±141.0) 17	13.8 (±5.5) 57
	F	16.3 (±1.8) 18	16.2 (±1.8) 6	31.9 (±23.6) 18	2.0 (±0.9) 18	61.1 (±5.2) 6	2.9 (±0.9) 6	370.7 (±381.4) 18	950.9 (±94.4) 6	10.3 (±3.2) 12
New York	M	28.4 (±3.9) 2	---	46.3 (±27.4) 2	2.9 (±0.3) 2	---	---	428.9 (±300.8) 2	---	---
	F	20.7 (±3.0) 11	---	22.6 (±11.1) 11	1.6 (±0.4) 11	---	---	278.6 (±150.1) 11	---	13.2 (±5.2) 11
PBI/MAFF										
F_1 & BC_1	M	22.4 (±1.6) 7	22.0 (±0.4) 2	32.9 (±18.2) 7	2.0 (±0.6) 7	56.7 (±8.5) 2	2.7 (±0.4) 2	368.5 (±206.3) 7	642.2 (±85.9) 2	13.2 (±5.1) 5
	F	21.8 (±3.6) 20	22.1 (±3.5) 17	52.2 (±16.0) 20	2.5 (±0.7) 20	56.0 (±13.4) 17	2.6 (±0.6) 17	605.8 (±172.4) 20	639.4 (±140.1) 17	16.7 (±4.7) 3

* Standard Deviation; ** Sample Size

Tables 3 and 4 separate the warfarin resistant PBI/MAFF test group by sex and by first filial (F_1) and back-cross (BC_1) generations. When the toxicity test results are examined for each sample by sex (Table 2) most of the results were not significantly different. Certain trends were observed, for instance, mean weights of males are greater than those of females. Males from the Ontario and New York samples ate more feed, had a larger mean warfarin dose therefore, and died on the average later in the test period than did females. Females from the resistant F_1 and BC_1 sample, on the other hand, ate more per day, for the most part, and received an overall higher mean warfarin dose than did males. There was little difference between resistant survivors within this test sample in terms of amount eaten per day and warfarin dose. Females within this sample generally died later than males.

Tables 3 and 4 show that both male F_1 and BC_1 outcross PBI/MAFF animals did not weigh more than females of the same type. This result is consistant with those observed in wild mice from Ontario and New York. Unlike the wild mice however, F_1 and BC_1 females ate more food in total, ate more food per day, and had a higher mean warfarin dose than did males. The finding that females in this sample consumed more warfarin than males did not change if the total sample was considered or whether animals which survived the toxicity tests were considered separately.

Weight Distribution

The animals of each geographic group were classified according to

Table 3: Mean weight of animals and mean results of toxicity testing with warfarin for the total test group and the warfarin resistant subgroup

Sample		Sample Size Total	Animal Weight (gm.)	Weight (Resistant Animals) (gm.)	Total Food Consumed (gm.)	Food Consumed Per Day (gm.)
PBI/ MAFF F_1		20	22.2 (±2.9) ** 20***	22.5 (±2.9) 12	41.9 (±16.8) 20	.2.2 (±0.6) 20
	M		22.6 (±1.9) 5		23.3 (±9.0) 5	1.8 (±0.4) 5
	F		22.1 (±3.2) 15	22.7 (±2.9) 12	48.1 (±14.1) 15	2.3 (±0.6) 15
PBI/ MAFF BC_1 *		7	21.1 (±4.0) 7		62.4 (±14.1) 7	3.0 (±0.7) 7
	M	2	22.0 (±0.4) 2		56.7 (±8.5) 2	2.7 (±0.4) 2
	F	5	20.7 (±4.8) 5		64.7 (±16.0) 5	3.1 (±0.8) 5

* All animals survived, WR values same as for whole sample.

** Standard deviation

*** Sample size

Table 4: Mean results of toxicity testing with warfarin for the total test group and the warfarin resistant group

Sample	Total Food Consumed (WR) (gm.)	Food Consumed Per Day/WR (gm.)	Mean Warfarin Dose (mg/kg.)	Mean Warfarin Dose/WR (mg/kg.)	Mean Day of Death
PBI/MAFF F_1	52.39** (±11.0) 12***	2.5 (±0.5) 12	475.8 (±190.0) 20	581.13 (±121.9) 12	14.5 (±5.0) 8
M			259.1 (±97.8) 5		13.2 (±5.1) 5
F	52.4 (±11.0) 12	2.5 (±0.5) 12	548.0 (±158.6) 15	581.1 (±121.9) 12	16.7 (±4.7) 3
PBI/MAFF BC_1*			74.0 (±87.9) 7		
M			642.2 (±85.9) 2		
F			779.1 (±55.2) 5		

* All animals survived, WR values same as for whole sample.

** Standard deviation

*** Sample size

weight (Table 35, Appendix A). Most of the mice in the Ontario samples fell between 11 and 20 grams whereas the New York wild mice and the PBI/MAFF resistant control mice and their descendents fell between 24 and 25 grams. No correlation was observed between animal weight and survival of the toxicity test in any of the groups.

Electrophoretic Results

Electrophoretic data (Tables 34 and 36, Appendix A) were examined for each test group as a whole, and for the group of animals which survived toxicity testing. As stated previously, no genetic variability was detected at the Alb-1 locus, but both alleles were detected at each of the Hbb and Gpi-1 loci for each test sample. Analysis by X^2 testing revealed no unusual allele distributions. The proportion of animals of a specific phenotype did not significantly differ between the test sample as a whole and the resistant sub-groups. Also, phenotypic distributions did not differ significantly in males and females.

The small test sample size used from New York State complicated the analysis of data. While no X^2 analyses could be performed, in the sample as a whole, all three known phenotypes controlled by each of the Hbb and Gpi-1 loci were expressed.

The known resistant PBI/MAFF test sample animals were monomorphic for the Hbb^s and $Gpi\text{-}1^b$ alleles.

This result is not totally unexpected because the origianl PBI mouse colony at Cambridge was rather small, and the outcross breeding

done in the Windsor laboratory was performed with inbred C57BL/6J mice which are homozygous for these two alleles.

Correlation Analysis of Toxicity Test Parameters

Correlation analysis was utilized to examine the toxicity data for any possible correlations between parameters. Table 5 presents the correlation analysis findings for each test sample as a whole and for the warfarin resistant animal group within each test sample.

In the total Ontario sample, significant positive correlations suggest, the heavier the animal, the more it ate per day, and in total, and the greater was the amount of warfarin consumed. Also, heavier animals died later in the test than did lighter ones. Animals which ate more warfarin-food mixture per day or in total died later than those which ate little. This last correlation likely reflects the fact, that some animals ceased to consume appreciable amounts of the warfarin-food mixture early in the test period, and of these animals, a small number may have starved to death. This point shall be discussed later in the section on autopsy findings. Death by starvation should occur before the maximum effects of warfarin poisoning would be observed in animals which consumed significant amounts of warfarin-food mixture.

In the animals from Ontario which survived the toxicity test, there was a significant positive correlation between animal weight and the amount eaten per day and in total, but there was a significant

Table 5: Correlation analysis of toxicity test parameters

Sample	Weight VS Amt. Eaten Per Day	Weight VS Total Amt. Eaten	Weight VS Day Dead	Weight VS Warfarin Dose (mg/kg)	Amount Eaten Per Day VS Day Dead	Total Amount Eaten VS Day Dead
Ontario Wild	0.40** *** 4.14 **** 92	0.30** 3.00 92	0.16** 1.33 69	0.07** 0.66 92	0.44** 4.01 69	0.87** 14.44 69
Survived Toxicity Testing	0.64** 3.99 25	0.64** 3.82 23		-0.41** -2.06 23		
New York Wild	0.57** 2.30 13	0.28** 0.97 13	-0.41** -1.35 11	-0.08NS -0.27 13	-0.29** -0.91 11	0.72** 3.11 11
Survived* Toxicity Testing						
PBI/MAFF F_1 & BC_1	0.40** 5.46 27	0.22** 1.13 27	-0.65** -2.10 8	-0.23** -1.18 27	-0.08NS -0.20 8	0.91** 5.38 8
Survived Toxicity Testing	0.47** 2.20 19	0.46** 2.37 19		0.70** 4.04 19		

* Only 2 animals survived, insufficient for analysis

** Significant at 1% level

*** t value

**** number of animals

negative correlation between weight and warfarin dose. These data shall be discussed further in subsequent sections.

As with the Ontario test sample, heavier mice in the New York sample ate more warfarin-food mixture. However, lighter mice died later than heavier mice during the toxicity test, an opposite finding to that of the Ontario sample. There was a significant positive correlation between total amount of food consumed and the day of death in the test period, but there was a significant negative correlation between the amount eaten per day and day of death. This result could be, because to eat a large amount of warfarin-food mixture, an animal must live a relatively long time within the test period; "long" life could best be achieved by eating a small amount of warfarin-food mixture per day, to sustain life, but preventing the warfarin dose from reaching lethal levels. There was no significant correlation between animals' weights and warfarin dose.

Because of small sample size, correlation analysis could not be performed utilizing the New York warfarin resistant animals.

Results of correlation analysis for the known-resistant outcross PBI/MAFF sample are similar to those obtained from the New York sample. However, unlike the New York sample, in the PBI/MAFF sample there was a significant negative correlation between animal weight and warfarin dose and there was no significant correlation between the amount of food eaten per day and day of death.

For the animals, within the PBI/MAFF sample, that survived the toxicity test, there were significant positive correlations between

animal weight and amount of food eaten per day, and in total, and warfarin dose.

Since all of these animals were supposed to be warfarin resistant, being at least heterozygous for the War gene (see the section on mode of inheritance in the Introduction), there are questions to be asked with regard to the animals which died.

If, as MacSwiney and Wallace (1978) propose that the War gene is controlled by modifiers, then outcrossing of the PBI mice could reduce expression of the gene and explain the mortality observed in the control animals. This reason, and the possibility that a few animals could have starved to death shall be discussed later.

Tables 6, 7 and 8 present similar correlation analyses to those presented in Table 5 however, the data for each test sample in these tables is presented by sex to try to illustrate potential sources of the differences in the test sample results observed in Table 5.

When the Ontario sample as a whole is examined (Table 6), there is a large difference between males and females. Females only show significant correlations between weight and amount eaten per day, between amount eaten per day and day of death, and between total amount eaten and day of death. The pattern of correlation coefficients for male and female resistant animals is the same as that obtained when sexes are combined.

A large difference is observed between linear regression results of the total test sample and females from the New York test sample. If only the females of this sample are examined, there is no significant correlation between weight of animal and amount of food eaten per day.

Table 6: Correlation analysis of toxicity test parameters

Sample & Sex		Weight VS Amt. Eaten Per Day	Weight VS Total Amt. Eaten	Weight VS Day Dead	Weight VS Warfarin Dose (mg/kg)	Amount Eaten Per Day VS Day Dead	Total Amt. Eaten VS Day Dead
Ontario Wild							
	M	0.41**	0.31**	0.13**	0.99**	0.38**	0.86**
		3.81*	3.30	0.97	0.77	3.05	12.50
		74***	74	57	74	57	57
	F	0.18**	0.06NS	-0.06NS	-0.08NS	0.63**	0.88**
		0.73	0.24	-0.19	-0.32	2.56	5.86
		18	18	12	18	12	12
Survived Toxicity Testing							
	M	0.56**	0.55**	---	-0.48**	---	---
		2.62	2.55		-2.12		
		17	17		17		
	F	0.59**	0.51**	---	-0.74**		
		1.32	1.18		-2.20		
		6	6		6		

* t value

** Significant at 1% level

*** Sample size

Table 7: Correlation analysis of toxicity test parameters

Sample & Sex	Weight VS Amt. Eaten Per Day	Weight VS Total Amt. Eaten	Weight VS Day Dead	Weight VS Warfarin Dose (mg/kg)	Amount Eaten Per Day VS Day Dead	Total Amount Eaten VS Day Dead
New York Wild						
M	---	---	---	---	---	---
F	0.14[NS]	0.06[NS]	-0.49[**]	-0.32[**]	-0.22[**]	0.79[**]
	0.42[***]	0.18	-1.59	-1.01	-0.64	4.07
	11[****]	11	10	11	10	10
Survived Toxicity Testing[*]						
M	---	---	---	---	---	---
F	---	---	---	---	---	---

* Insufficient number of animals (2) for analyses

** Significant 1% level

*** t value

**** Sample size

Table 8: Correlation analysis of toxicity test parameters

Sample & Sex		Weight VS Amt. Eaten Per Day	Weight VS Total Amt. Eaten	Weight VS Day Dead	Weight VS Warfarin Dose (mg/kg)	Amount Eaten Per Day VS Day Dead	Total Amt. Eaten VS Day Dead
PBI/ MAFF							
F_1 & BC_1	M	0.41**	-0.14NS	-0.41NS	-0.20NS	-0.41NS	0.89**
		1.00***	-0.32	-0.78	-0.46	-0.78	3.38
		7****	7	5	7	5	5
	F	0.47**	0.40**	-0.86**	-0.23**	0.67NS	0.95**
		2.26	1.85	-3.30	-1.00	0.90	3.04
		20	20	3	20	3	3
Survived* Toxicity Testing							
	M	---	---	---	---	---	---
	F	0.51**	0.46**	---	-0.30**	---	---
		2.30	2.01		-1.22		
		17	17		17		

* Insufficient number of animals (2) for analysis

** Significant 1% level

*** t value

**** Sample size

or in total, but there is a significant negative correlation between weight of animal and warfarin dose (mg/kg of body weight).

Results for other analyses remain unchanged if the small male group is excluded from the calculations.

For the PBI/MAFF sample as a whole, results are non-significant for males except between weight of animals and amount eaten per day and also between total amount eaten and day dead. Results for all females in the test sample do not differ from those obtained when both sexes are examined together (Table 5). When females that survived the toxicity tests are examined, results similar to those observed for the Ontario sample are obtained. There is a significant negative correlation between animal weight and warfarin dose. These results illustrate the effect that several heavy males can have on correlation analysis of a sample consisting of both sexes. Correlations may switch sign and/or become significant as compared to analyses on the sexes when considered separately. This phenomenon is particularly true for analyses of small sample sizes.

Hemorrhage Findings

Tables 9 and 10 present a summary of hemorrhage findings in the test animals before death and at the time of autopsy. The presence of hemorrhages at the time of death is a good indication that the animal suffered effects of warfarin. Detailed findings for each animal are presented in Table 37, Appendix A.

Of the animals which died, 17.3 percent showed external bleeding at least one day before death occurred. The most common site of

Table 9: Summary of hemmorhage findings before death and at autopsy. In scale of severity, 1 is mildest and 3 is most severe.

Sex & Total	Before Death	External at Death	Abdomen Severity 1	2	3	Sum	G.I. Tract Severity 1	2	3	Sum
Males N = 58	12 (85.71)*	12 (60.00)	35 (74.47)	3 (75.0)	17 (73.91)	55 (74.32)	15 (65.22)	2 (50)	6 (66.67)	23 (63.89)
Females N = 23	2 (14.28)	8 (40.00)	12 (25.53)	1 (25.0)	6 (26.09)	19 (25.68)	8 (34.78)	2 (50)	3 (33.3)	13 (36.11)
Total N = 81	14 (17.28)	20 (24.69)	47	4	23	74 (91.36)	23	4	9	36 (44.44)

* Percent of N

Table 10: Summary of hemmorhage findings before death and at autopsy. In scale of severity, 1 is mildest and 3 is most severe.

Sex & Total	Before Death	External at Death	Thorax Severity 1	2	3	Sum
Males N = 58	12 (85.71)*	12 (60.00)	10 (90.9)	1 (50)	2 (66.67)	13 (81.25)
Females N = 23	2 (14.28)	8 (40.00)	1 (9.1)	1 (50)	1 (33.33)	3 (18.75)
Total N = 81	14 (17.28)	20 (24.69)	11	2	3	16 (19.75)

* Percentage of N

external bleeding was the facial region, but some bleeding of the distal phalanges also occurred. At the time of death, 24.7 percent of animals showed signs of external bleeding. Internal bleeding was recorded at autopsy, being limited to hemorrhages easily visible to the naked eye on examination of the abdominal or peritoneal cavity, the gastro-intestinal lumen, and the thoracic cavity. Severity of the hemorrhage is indicated by a subjective, numerical scale, 1 being a slight hemorrhage, and 3 being a large pool of blood located in the particular area within the mouse. At the time of autopsy, 91.4 percent of animals exhibited abdominal cavity hemorrhages, 44.4 percent had bleeding within the gastro-intestinal lumen, and 19.8 percent had bleeding within the thoracic cavity. Thirty-nine (48.1 percent) of animals exhibited bleeding at more than one location. Five animals (6.2 percent) showed no signs of bleeding before death or at the time of death. Of these five animals however, only two died before day 10 of the test and death could have been due to starvation and not hemorrhaging except that one mouse ate 2.2 grams of food per day, which is considered adequate to maintain life. The other animal ate 0.7 grams of food per day. Another mouse ate less than 2.0 grams, but showed hemorrhages, so death could have been the result of a combination of hemorrhage and starvation. Few animals, therefore, appeared to have starved to death. Hemorrhage due to warfarin consumption was probably the major cause of death.

Warfarin Loading Dose

In mammalian species, a "loading" period is required to produce optimal anticoagulant effects with warfarin (Goodman and Gilman, 1975). Loading period refers to the period of several days over which warfarin must be consumed, in at least therapeutic dosage, to produce anticoagulant effects as determined by at least a 25 percent increase in prothrombin time.

In the present study, C57BL/6J animals were administered a single large dose of warfarin, by gavage, in order to determine if such a dose is lethal (Table 11). If animals survived, they would provide further evidence that the metabolic pathway for warfarin can detoxify large amounts of the drug as long as it is not presented chronically. This shall be discussed later. Male and female inbred mice were given either 20, 40 or 80 mg/kg of body weight of sodium warfarin in distilled water. A dose of 20 mg/kg of body weight is approximately equivalent to the consumption of 2000 grams of 0.025 percent warfarin-feed mixture. Five control animals of each sex were given distilled water by gavage, none showed any ill effects. In the three tests, only one male and one female died, both having received 80 mg/kg of body weight of warfarin. Neither of the dead animals showed substantial hemorrhages.

Estimated Frequency of Resistance Allele

Based on a two allele with dominance situation, the frequency of the allele responsible for resistance (War?) was estimated in the

Table 11: Examination of loading dose of sodium warfarin

Dose	20 mg/kg.			40 mg/kg.			80 mg/kg.		
Sex	M	F	Total	M	F	Total	M	F	Total
Number Animals	5	5	10	6	6	12	5	5	10
Mean Weight Treat #1 (gm)	26.4 (±2.6)*	22.6 (±3.5)	24.5 (±3.5)	27.2 (±4.5)	23.6 (±2.7)	25.4 (±4.0)	26.0 (±2.0)	21.9 (±1.3)	24.0 (±2.7)
Mean Weight Treat #2 (gm)	26.4 (±2.1)	24.5 (±2.0)	25.4 (±2.2)	27.2 (±4.6)	23.5 (±2.9)	25.4 (±4.1)	26.2 (±2.3)	22.1 (±1.5)	24.2 (±2.7)
Number Dead	0	0	0	0	0	0	1	1	2
Weight Dead							24.9	21.1	---

* Standard deviation

Ontario and New York wild populations and in British populations (Table 12) (data from Rowe and Redfern, 1965). The frequency of the resistance allele was 0.14 in Ontario, and 0.08 in New York populations. From a pooled sample of unexposed and suspected resistant British populations the frequency of the alleles was 0.18. In both the Ontario and British populations, females had a higher frequency of the allele, than males.

Teratology

Animal studies have shown that warfarin may exert toxic effects in pregnant animals also. In the present study a small number of C57BL/6J and C3H mice (10 and 6 respectively) were treated with 4.5 mg/kg of body weight of sodium warfarin by gavage on gestation days 7 to 10. Sodium warfarin was dissolved in distilled water. The dose utilized was the median effective dose determined from data presented by Kronick et al. (1974). Gestation days 7 to 10 were determined from plugs observed on day 0 after timed matings. Autopsy was performed on day 18. In no case, was any malformed animal obtained from litters (N = 104, 16 litters).

Discussion

Comparison of Variability in Response to Warfarin in British and North American Populations of Mus musculus

Rowe and Redfern (1964, 1965) developed and utilized a warfarin toxicity test, described in Methods, to screen wild-caught British

Table 12: Estimated frequencies of War? in natural populations of Mus musculus

Source	Mortality	Allele Frequency	Pooled
A) Rowe and Redfern (1965)			
Unexposed Populations	48/53 (90.6%)	p = 0.048	p = 0.063
	31/37 (83.8%)	p = 0.084	
Suspected Resistant Populations	22/52 (42.3%)	p = 0.350	p = 0.300
	32/56 (57.1%)	p = 0.244	
males	13/26 (50.0%)	p = 0.207	p = 0.258
	14/23 (60.9%)	p = 0.220	
females	9/26 (34.6%)	p = 0.412	p = 0.324
	18/33 (54.5%)	p = 0.261	
B) Ontario	69/94 (73.4%)		p = 0.143
males	57/74 (77.0%)	p = 0.122	
females	12/18 (66.7%)	p = 0.184	
C) New York	11/13 (84.6%)		p = 0.080
males	1/2 (50.0%)	p = 0.293	
females	10/11 (90.9%)	p = 0.046	

Mus musculus for warfarin resistance. The application of this procedure to wild mice from corn cribs in Ontario and from farm buildings in New York State revealed resistance to large amounts of warfarin. This is consistant with the findings of Rowe and Redfern in Britain.

Furthermore, considerable variation in the susceptibility of individual mice was observed in both studies. In Rowe and Redfern's study, the first death occurred on the third day of the test. In the present study, the earliest death observed occurred on day 4. The lowest fatal dose in Rowe and Redfern's (1964) study was 26.1 mg/kg of body weight for a 13.4 gram male which ate 1.4 grams of warfarin-food mixture over a 4 day period, and showed normal warfarin symptoms. The largest dose survived was equivalent to 1067.2 mg/kg of body weight for a 11.9 gram female which ate 50.8 grams over a 21 day period. A similar range of values was obtained in Rowe and Redfern's (1965) study. In the present study the lowest fatal dose was 28.4 mg/kg of body weight for a 17.6 gram female which ate 2.0 grams of the warfarin diet and died on day 7 (only a slight hemorrhage was observed) and the largest dose survived was that of 1250.0 mg/kg of body weight for a 15.2 gram male which ate 76.5 grams over a 21 day period. Two animals maintained on the warfarin test diet for 42 days had equivalent warfarin doses of 2389.5 and 2445.1 mg/kg of body weight respectively.

As in Rowe and Redfern's study, warfarin resistant mice were observed in nearly all the populations sampled. In the Ontario

samples, resistance was found in 26.6 percent of animals tested, but perhaps due to the small sample sizes from some populations no significant differences were detected among populations. While relatively few females were available for toxicity testing in the Ontario sample (because of the pre- and post-implantation mortality studies), a slight difference in susceptibility between the sexes was observed. Of the males used, 25.3 percent (19/76) survived the toxicity tests and 33.3 percent (6/18) of the females survived. This greater proportion of surviving females was also observed by Rowe and Redfern (1964). Differences in susceptibility to warfarin in males and females could be due to the sex dependent penetrance properties of War observed by Wallace.

No obvious differences in susceptibility to warfarin existed based on a comparison of mean weights of animals. Males had larger mean weights than females, but there was no significant difference between male and female test groups which were found susceptible or resistant to warfarin.

In Tables 9 and 10, data were presented which showed that many animals (17.3 percent) showed signs of hemorrhage before the time of death. Some animals, showed signs of illness, such as weakness or lethargy, at some time during the test and ate only sparingly for several days before recovering and eating normally. These findings are also similar to those obtained by Rowe and Redfern (1964).

Autopsy of animals suspected to have died from warfarin poisoning revealed that hemorrhages could occur at a number of sites within the

body, and could occur at several sites concurrently. This finding agrees with that obtained by Bonnet et al. (1951). In the present study the most frequent sites of hemorrhaging were the abdominal cavity (91.4 percent), the gastro-intestinal lumen (44.4 percent) and the thoracic cavity and organs (19.8 percent). The central nervous system was not examined for hemorrhages, but this does not appear to have been a major cause of death since no signs of paralysis or "stroke-like" symptoms were observed. The distribution of hemorrhages determined from this study differs from that found by Rowe and Redfern (1964) where the thoracic cavity was the most common hemorrhage site followed by the digestive organs, central nervous system, lungs and abdominal cavity.

As in Rowe and Redfern's (1964) study, most animals in the present study lost weight during toxicity testing. The decrease in body weight in most cases was approximately 20 percent. Animals which survived toxicity testing generally lost less body weight than those animals which died. Weight loss was not limited to the initial part of the testing period.

The last test animal to die did so on the 26th day after the warfarin diet was first presented (the animal was placed on a feed-mixture diet on day 21). While this result is shorter than that determined by Rowe and Redfern (1965), day 64, the effects of warfarin in mice can be quite prolonged.

In summary, the findings, in regard to the percentage of animals which survived toxicity testing, of the present study are within the

range established by Rowe and Redfern (1964, 1965) for wild Mus musculus populations in Britain. In this study 26.6 percent of wild Ontario sample mice, 15.4 percent of New York sample mice and 70.4 of outcross resistant mice survived toxicity testing. Rowe and Redfern found that 9.4 to 16.2 percent of animals from populations unexposed to warfarin survived toxicity testing and 42.8 to 57.7 percent of animals from sites where warfarin had been used as a rodenticide survived toxicity testing. It is known that warfarin had been used previously at one trapping site in the Ontario sample and at several sites for the New York test sample. Animals were found to be resistant at these locations. Although it was not established that warfarin resistance in North American Mus musculus populations was due to the same allele at the same locus, this was assumed to be the case in order to compare gene frequencies in these populations with those in Britain. The frequency of the resistance allele (War?) in the Ontario populations (0.14) is very similar to the value mean (0.18) calculated from the data presented in Rowe and Redfern's studies (1964, 1965). The frequency obtained from the very small New York sample (0.08) was in the lower end of the range (0.06) which Rowe and Redfern (1965) found in populations unexposed to warfarin. These findings are very interesting in that if the mode of inheritance is similar on both continents, then the amount of genetic variability is also similar. It appears as though some selective force could be maintaining a stable polymorphism for War at this relatively low frequency. The presence of this polymorphism indicates that resistance to warfarin is a common occurrence

in mice, even in those animals which are geographically isolated.

While there was little difference in the proportion of animals which survived toxicity testing between the present study and that of Rowe and Redfern, the mean day of death in all three test samples in this study was greater than that obtained by Rowe and Redfern (1964). The Ontario test sample had a mean day of death of 13.2 $\pm$ 5.3, the New York sample had a mean day of death of 12.9 $\pm$ 5.0 and the resistant outcross sample had a mean day of death of 14.5 $\pm$ 5.0. These values are larger than those obtained by Rowe and Redfern for their unexposed sample (6.3 $\pm$ 2.2) and their suspected resistant animals (11.7 $\pm$ 7.7 and 11.9 $\pm$ 6.1).

Electrophoretic examination of phenotypes at the Hbb and Gpi-1 polymorphic loci linked to War and at the Alb-1 monomorphic serum albumin locus revealed no patterns or obvious differences in susceptibility to warfarin among the different possible genotypes. There appears to be no association therefore between these linked loci and the resistance phenotype.

Correlation analysis revealed a number of trends, and several possible inconsistencies. In all cases, if each test sample is considered as a whole (males and females) there is a significant positive correlation between animals' weight and the amount of warfarin-feed mixture consumed per day.

If these samples are broken down by sex to try to get more detailed information, this clear correlation changes to some degree. For example, in the Ontario sample there is no significant correlation

for female weight and total amount eaten per test period. The observed changes in correlation pattern may be due to several causes. Loss of significance in correlations dealing with female weight and toxicity parameters may result from the fact that males weigh more than females and make up a larger proportion of the animals within the total Ontario sample tested. In the PBI/MAFF test sample females outnumbered males, and it is in the latter sex that changes in correlation patterns from the total sample were observed. In general, due to weight differences between sexes and unequal, small sample sizes, the correlations obtained for the total test samples may be of greatest value in determining trends of association between toxicity parameters. Examination of components of the test samples may indicate some reasons for the observed inter-test sample variation, however.

No clear trends of association existed between how much an animal weighed or ate per day and when or if it died during the toxicity period.

That there was a general trend in the study for large animals to eat more but differences in the direction of the correlation between animal weight and warfarin dose in the Ontario and outcross PBI/MAFF test samples, may again reflect differences in the male/female proportions in the two test samples.

As would be expected, the total amount of food consumed was significantly positively correlated with day of death. In order to eat a large amount of poison-feed mixture, an animal would likely have to live for a relatively long period of time, since no animals consumed

large amounts of food at any one time.

As described in the Introduction, the penetrance of the gene War is affected by age and sex and has one or more modifiers. These properties of the gene may be utilized in examining the results of this study, if it is assumed that the same type of inheritance and the same alleles are involved. Rowe and Redfern (1967) showed that young males (less than 4 months) show the same resistance patterns as females, and that penetrance in males decreases with advanced age. In the present study, all animals were more than four months old, but wild males could easily have been a year old at the time of the study. Advanced age in these males could account for some of the deficiency of resistant males found in the wild samples. Differences in the percent survival of wild animals from New York and Ontario could also be due to differences in the modifier complexes of the two samples' genetic backgrounds. Differences in modifier complexes may also be reflected in survival differences between F_1 and BC_1 outcross PBI/MAFF control animals. All of these animals should theoretically have survived since they were all at least heterozygous for War. The fact that only 60 percent (12/20) of the F_1 animals while 100 percent (7/7) BC_1 animals survived, could be explained by BC_1 animals having a greater dose of resistance modifiers than the F_1 animals.

The hypothesis of Bishop et al. (1977) and Greaves et al. (1977) that warfarin resistance in R. norvegicus from Wales is maintained as a balanced polymorphism by selection could possibly have relevance for

wild mice also. The authors propose, that selection against susceptible homozygotes by the use of warfarin and selection against resistant homozygotes because of high vitamin K_1 requirements gives rats heterozygous for the $\underline{Rw^1}$ and $\underline{Rw^2}$ alleles a selective advantage. If the same situation exists for wild mice, this hypothesis would help to explain why the frequency of War? appears to be so similar in wild British and North American populations of Mus musculus.

Further work on this topic should include mapping and linkage studies on natural populations of Mus from North America. Also to be done are tests to determine if in fact all populations carry the same allele for resistance. Finally, the mechanism by which warfarin resistance occurs must be more fully elucidated.

Summary

As in British populations, variability in response to warfarin exists in North American populations of Mus musculus. If the mode of inheritance is similar in both groups of populations, then the frequency for the resistance allele is similar in Britain (0.18) and Ontario (0.14). There appears to be no association between the loci Hbb and Gpi-1, which are linked to War, and the resistance phenotype. It is not possible to predict from an animal's weight or sex whether an animal is resistant to warfarin or not.

CHAPTER III

VARIABILITY IN BLOOD CLOTTING AS DETERMINED BY PROTHROMBIN TIME MEASUREMENT IN INBRED AND WILD *MUS MUSCULUS*

The Quick One-Stage Prothrombin Time Test is often utilized to measure blood clotting time and to monitor the anticoagulative effects of warfarin on blood (Quick, 1966).

Differences in prothrombin times among different individuals should reflect concentration differences in blood clotting factors. Clotting factors VII and/or X, both of which are vitamin K dependent and are affected by warfarin, do exhibit concentration differences detectable by the Quick Test.

The purpose of this study was to determine if variation in blood clotting times, as determined by the Prothrombin Time Test, existed in inbred and wild house mice. It was hoped that any variability found could be utilized as an indicator for warfarin resistance. Modifications of Quick's Method were attempted to improve its resolution. Finally, warfarin lengthened prothrombin times were determined and compared in inbred and wild *Mus musculus*.

Material and Methods

Source of Animals

Inbred animals utilized for this study were obtained from laboratory bred stocks descended from mice obtained from the Jackson

Laboratory, Bar Harbor, Maine. As described earlier, in the chapter on Toxicity Testing, mice from natural populations were trapped in south-western Ontario and obtained from samples trapped by A. Miller-Baker near Stoneybrook, New York. Warfarin resistant and susceptible animals utilized were obtained from M.E. Wallace, at Cambridge University, and J.H. Greaves, Hook Rise South Laboratory, Surrey, England. These animals have also been described previously.

Prothrombin Time Test Procedure

Siliconized capillary tubes were used to withdraw 400 μl of blood from the sub-orbital sinus. The blood was placed in siliconized 10 x 75 mm tubes containing 0.1 ml sodium citrate (5 percent sodium citrate in mammalian physiological saline - 0.8 percent NaCl). All sample tubes were kept on ice. Blood was prepared for testing by centrifugation for 4 minutes at approximately 220 g.

For coagulation times, two sets of reagents were utilized. Bacto-Thromboplastin was obtained from Difco Laboratories (Detroit, Michigan) and was prepared for use in prothrombin time testing as directed by the manufacturer.

Simplastin (lot #2298033), abnormal citrate "verify I" plasma and "verify II" plasma (lots #2463043 and 2643063) came from Warner Lambert (Morris Plains, New York) and Ortho Plasma (lot #11R439) was produced by Ortho Pharmaceutical (Don Mills, Ontario).

To confirm the reliability of the test method, parallel tests, with Thromboplastin and Simplastin were done on human plasma (Ortho-Plasma),

abnormal human plasma (Citrate "verify I" and "verify II") and inbred mouse plasma.

Prothrombin times were obtained by the Quick One-Stage Tilt Method as described by Quick (1966) and in the Difco Bacto-Thromboplastin instructions. Final prothrombin time was based on at least two replicates of each test sample and the times were recorded to one-tenth of a second.

All solutions used for the test procedure were prepared with glass distilled water.

In this study, sodium citrate rather than sodium oxalate was used to remove calcium in the preparation of blood for the Quick Test. This was necessary to reduce erythrocytic lysis during blood processing prior to testing.

All calculations of data were performed on a Monroe 1860 calculator.

Results

A summary of mean prothrombin times for all groups of animals examined is presented in Table 13. The range of values for all groups was between 10.0 seconds and 19.3 seconds, and the range of mean values per group for all test groups was 11.9 seconds to 16.4 seconds. Mean prothrombin time for all test groups was 14.3 ± 1.4 seconds; the inbred strains had a mean prothrombin time of 14.0 ± 1.5 seconds; the "wild" mice and descendents of wild mice had a mean time

Table 13: Summary of mean prothrombin times per test group

Animals		Number of Animals	Mean Prothrombin Time (sec.)
Inbred			
C3H		10	15.4 ± 1.0*
C57BL/6J		12	15.6 ± 1.3
C3H x C57 F_1		12	14.7 ± 1.4
C3H x C57 F_2		14	12.6 ± 1.8
C3H x C57 BC_1		15	12.9 ± 2.1
CBA		8	12.7 ± 0.7
DBA		5	13.7 ± 2.3
A/J		9	16.2 ± 1.4
Total		85	14.2 ± 1.4
Non-Inbred			
Brachy		5	11.9 ± 0.5
Descendants of British	Warfarin Resistant	8	14.1 ± 0.3
Wild Caught	Warfarin Susceptible	7	13.6 ± 0.5
Wild Caught	Ontario Wild	13	14.6 ± 1.3
	New York Wild	10	15.3 ± 1.1
	Ontario Wild (Warfarin Resistant)	10	16.4 ± 1.4
Total		53	14.3 ± 1.5
Total Inbred and Non-Inbred		138	14.3 ± 1.4
Total Non-Inbred Resistant		18	15.2 ± 1.6

* Standard Deviation

of 14.8 ± 1.1 seconds; and the resistant animals, both from the outcross PBI x C57BL/6J (warfarin resistant) and from the natural population of Ontario had a pooled mean prothrombin time of 15.2 ± 1.6 seconds.

While the warfarin resistant animals have a greater mean time than inbred and susceptible animals, the difference is not significant, and moreover, there are several test groups which have individual mice with longer times than that of the resistant animals. The various mean values are very similar to a previously reported value of 14.9 ± 3.2 seconds for inbred mice (Meier et al., 1961) and they fall within the range that is reported for normal humans, 11 to 15 seconds (Quick, 1966; Faulkner and King, 1979). These results shall be discussed further later.

Within the replicates of a single sample prothrombin time values were within a one second range.

Table 14 summarizes the comparison of prothrombin times as determined by two slightly different methods. Simplastin, is a premixed solution containing both thromboplastin and calcium chloride. Bacto-Thromboplastin does not contain calcium chloride which therefore must be added separately to the test serum-thromboplastin mixture.

Within either of the two test methods, there was little difference in mean prothrombin times for inbred C3H and C57BL/6J plasma. Bacto-Thromboplastin produced slightly longer mean values than did Simplastin. When human control plasma (Ortho) was used instead of mouse plasma, a difference of only 0.2 seconds was found. This is well

Table 14: Comparison of two methods of prothrombin time measurement

Serum Source	MEAN PROTHROMBIN TIME (Sec.) Simplastin (Thromboplastin & Ca^{++}) 2.0 ml	Difco Thromboplastin & Ca^{++} 2.0 ml
C3H	12.9 ± 0.4*	17.3 ± 0.3
C57BL/6J	13.6 ± 0.4	15.9 ± 0.1
Ortho Control Plasma	16.4 ± 0.6	16.2 ± 0.2
Verify Abnormal Citrate I	22.4 ± 0.3	17.9 ± 0.6
Verify Abnormal Citrate II	32.1 ± 0.2	24.4 ± 0.4

* Standard Deviation

within the 4.0 second range considered acceptable between the two methods. Abnormal plasmas (Citrates "verify I" and "verify II") produced longer prothrombin times than the plasmas from other sources, as expected. For the abnormal plasmas, however, Difco Bacto-Thromboplastin produced shorter mean prothrombin times than did Simplastin.

In summary, both sets of reagents used in prothrombin time measurements have very similar resolving powers.

Because preliminary studies suggested significant differences in prothrombin times of C3H and C57BL/6J inbred mice plasmas, an attempt was made to increase resolving power by altering conditions of the test by a) using various concentrations of sodium citrate, b) adding physiological or mouse buffered saline to the sodium citrate and c) maintaining the Thromboplastin at 37°C for 2 hours instead of 5 minutes after thawing before use. Results of these modifications are presented in Table 15. When mouse buffered saline instead of physiological saline is utilized to make the sodium citrate solution, there is a decrease in the mean prothrombin time for both C57BL/6J and C3H mice. If the concentration of sodium citrate utilized is increased, there is an increase in mean prothrombin times for both strains. Finally, when frozen Bacto-Thromboplastin is thawed and incubated for 2 hours at 37°C instead of 5 minutes, again plasmas of both strains react the same with a slight increase in mean prothrombin time.

Lengthened prothrombin time with increased concentration of sodium

Table 15: Effects of test system modification on prothrombin test

Test Condition	Mouse Strain: C3H		Mouse Strain: C57BL/6J	
Sodium Citrate/ Physiol Saline	10*	15.4** ± 1.0***	10	15.6 ± 1.3
Sodium Citrate/ Mouse Buffered Saline	5	11.8 ± 0.8	5	12.6 ± 1.4
10.0 Percent Sodium Citrate	6	55.8 ± 3.4	6	50.9 ± 10.0
7.0 Percent Sodium Citrate	4	19.4 ± 1.3	3	21.4 ± 0.9
5.5 Percent Sodium Citrate	2	15.6 ± 0.0	4	19.4 ± 19.4
Thromboplastin Thawed 2 Hours 37°C	3	17.9 ± 4.9	3	19.8 ± 1.3

* Number of animals
** Prothrombin time (sec.)
*** Standard deviation

citrate was likely due to excessive chelating potential, even after recalcification of plasma. Thromboplastin was active after storage at 37°C for two hours but since it is known, that the brain extract is stable at room temperature for up to six hours, two hours at only moderately higher temperatures does not seem unreasonable. Therefore under the conditions used, no significant difference in prothrombin time resolution was obtained in the two strains.

The effects of warfarin ingestion on prothrombin times are presented in Table 16. Mice from natural populations were fed a diet containing 0.025 percent warfarin for the purpose of screening for warfarin resistance (see chapter on Toxicity Testing). Prothrombin time test values were determined for apparently healthy animals after they had been on the warfarin diet for at least 10 days. The mean prothrombin time values increased from 14.6 ± 1.3 seconds and 15.3 ± 1.1 seconds to 57.3 ± 3.6 and 55.0 ± 7.9 seconds for Ontario and New York animals respectively.

Inbred C3H and C57BL/6J animals utilized for a loading-dose toxicity test (see chapter on Toxicity Testing) had a slightly lower mean prothrombin time than the wild animals, the value in this case changing from 15.4 ± 1.1 seconds to 54.5 ± 6.4 seconds.

Warfarin treatment of mice therefore produces prolonged prothrombin times. The values obtained in the present study are somewhat longer than those reported by Kronick et al. (1974) who found that when F_1 C3H x A/J pregnant mice were given 4 mg/kg of body weight of warfarin for a period of 9 days a mean prothrombin time of 48.9

Table 16: Prothrombin times for animals ingesting sodium warfarin

Animal Source	Number of Animals Before Ingestion	Number of Animals After Ingestion	Prothrombin Times Before Sodium Warfarin Ingestion (Sec.)	Prothrombin Times After Sodium Warfarin Ingestion (Sec.)
Ontario Wild Caught (21 Day Toxicity Test)	10	10	16.4 ± 1.4*	57.3 ± 3.6
New York Wild Caught (21 Day Toxicity Test)	10	10	15.3 ± 1.1	55.0 ± 7.9
Inbred (C3H & C57BL/6J) (3 Day - Gavage Toxicity Test)	10	10	15.4 ± 1.1	54.5 ± 6.4
Total	30	30	15.7 ± 1.2	55.7 ± 6.1

* Standard deviation

seconds was obtained. The difference between the two sets of results does not appear significant, and likely is in part due to differences in doses and type of animal Kronick et al. used. The values obtained in the present study are also longer than those reported for warfarin treated humans, about 28 seconds (Seegers, 1967; Quick, 1966). This result is also likely explained by differences in administered doses.

Discussion

Since the range of normal prothrombin times in the present study is quite large, the values obtained for the different groups (see Table 13) likely do not represent any significant differences. Normal values are therefore comparable regardless of the sex, age and strain of mouse used. This is consistant with the results reported by Meier et al. (1961). Even animals having considerable genetic variability, such as those from natural populations, show no significant differences in clotting times.

Whether test plasma is recalcified at the same time as thromboplastin addition (Simplastin), or is recalcified separately Bacto-Thromboplastin, makes little difference in prothrombin time values. Control experiments using human plasma provided results very similar to those obtained with mouse plasma. Abnormal "citrate I" and "citrate II" plasmas which represent conditions found in humans treated with anticoagulants produce longer prothrombin times than control plasmas, but vary in prothrombin time. The warfarin resistance trait appears to

have no effect on prothrombin time. Times for warfarin resistant animals are very similar to those of other mice, both wild and inbred.

It makes little difference to prothrombin time values whether Simplastin or Thromboplastin reagents, with different sequences of recalcification, are utilized. Control experiments using human plasma provided results very similar to those obtained with mouse plasma. Abnormal citrate "verify I" and "verify II" plasmas, which represent conditions found in humans treated with anticoagulants produce longer prothrombin times than control plasmas, but that are shorter than values obtained with warfarin treated mice. These differences are to be expected however, since human therapeutic doses of warfarin are normally less than 4 mg/kg of body weight whereas the warfarin treated mice had received more than 20 mg/kg of body weight.

Changing prothrombin time test conditions changes mean times from those observed under normal conditions, but does not detect any difference between C3H and C57BL/6J inbred strains.

Summary

Prothrombin times were determined in inbred and wild mice according to Quick's Method. No significant differences were found within or between inbred and wild mice samples or between warfarin sensitive and resistant house mice. Modification of test procedures changed mean prothrombin times per test group, but did not improve the resolution of the testing procedure. Administration of warfarin to mice resulted in prolonged prothrombin times, as expected, but did not alter the difference in times between inbred or wild animals.

The normal clotting time range of 4 seconds for this particular procedure does not permit a rapid indication of warfarin sensitivity.

More sensitive procedures are required to detect variability in prothrombin times between individual mice. The Quick One-Stage Method while valuable in determining estimates of clotting time does not have the necessary resolving power.

CHAPTER IV

VARIABILITY IN PRE- AND POST-IMPLANTATION MORTALITY IN WILD *MUS MUSCULUS*

The decision to study pre- and post-implantation mortality in wild *Mus* was originally made to provide background information for a teratology study of the effects of warfarin on pregnant wild mice. Although such a teratology study could not be carried out due to difficulties in inducing mating of wild animals under laboratory conditions, this pre- and post-implantation study does provide background information on embryonic mortality and its variability in the populations examined.

A rapidly increasing population may possess the reproductive potential to more than replace its numbers. Such was the situation found in thirteen natural populations of the house mouse, *Mus musculus*, studied in south-western Ontario: 54.5 percent of the adult females collected were pregnant, with a mean litter size of 5.97 $\pm$ 0.24.

The present study examined pre- and post-implantation mortality and mortality variability in *Mus musculus*. Females came from natural populations with different environmental situations and were collected during different seasons. Correlation was sought between mortality and gestation period, embryonic position within the uterus, environmental factors, and maternal genotype at six loci. Results of the study gave information on the affects of certain stresses on prenatal mortality and also served as an indication of relative "genetic load" in the sampled populations. Many parameters examined overlapped with those

presented by Batten and Berry (1967) permitting therefore a comparison of Ontario and British Mus musculus populations.

Finally, in the process of this study, a comparison of macroscopic corpora lutea counts with those obtained from serial histological sections was made.

A brief review of literature dealing with pre- and post-implantation mortality will now be presented.

Embryonic Mortality Estimates:

As with all mammalian species, mortality in the mouse may occur at any stage of the life cycle. Mortality may involve the loss of an unfertilized ovocyte, the loss of pre-implanted zygote, loss of an implanted zygote or embryo before birth, or loss of an organism post-partem. In the present study, mortality only during the time period before parturition was examined.

Batten and Berry (1967) review the factors which determine the number of young to which a polytocous animal, such as the mouse gives birth. These factors include parental genotype, environmental stimuli and maternal-embryo interactions.

Both maternal and paternal genotypes are important. For instance, Falconer (1960) states that the number of eggs ovulated, in laboratory mice, is controlled multifactorially, being affected by overall maternal body size, which is in turn, regulated by a number of loci. Furthermore, Falconer showed that increased parental inbreeding decreased litter size and that ovulation was affected by parity of dam in

early litters. Similarly, Braden (1958) reported, that the efficiency of sperm penetration of ova varied among inbred strains and Lewontin and Dunn (1960) in their studies of the t alleles found an association between these alleles, and sperm function.

Implantation may be prevented by abnormal cleavage of the zygote (Braden, 1958) or by changes in the uterine environment induced by such stimuli as crowding stress or perhaps even the behavioural-endocrine feedback system proposed by Christian and Davis (1964).

Once the zygote is successfully implanted, normal development may be hindered by deleterious genetic factors within the embryo itself (Lyon, 1959) such as chromosomal aberrations, by changes in the uterine environment brought about by factors such as those associated with increased maternal age and maternal stress, and by environmental insults such as climatic differences (Biggers et al., 1958) and by teratogens.

Several of these factors may be involved simultaneously, in the determination of individual and whole litter survival, and in many cases, it is impossible to ascertain the exact cause of prenatal mortality. Since the prime cause of mortality cannot be established, emphasis in the study was on the amount of mortality rather than its causes. Estimates of prenatal mortality have been determined for a number of different organisms.

Batten and Berry (1967) reviewed prenatal mortality in laboratory mice, and wild rats. Pre-implantation loss varied between 9.7 percent in DBA mice and 42.8 percent in F_1 offspring of CBA males and 101

females. Estimates of total prenatal losses for inbred mice vary between 10.5 percent in F_1 offspring of 101 males and C3H females to 47.8 percent for F_1 resulting from matings of CBA males by 101 females.

From wild mice caught in corn cribs and barns in northern Britain, Batten and Berry (1967) estimated that the total prenatal loss was between 31.1 percent and 36.2 percent, depending on the habitat examined.

In wild rabbits, Brambell (1948) determined that the total loss of ovulated ova was 43.3 percent of which 10.2 percent was pre-implantation. Most of the remaining loss occurred before the mid-point of gestation.

Adams (1960) used laboratory rabbits to show that pre-implantation ovum loss was about 11.4 percent, while after implantation, the equivalent of 18.3 percent of ova were lost.

Carr (1977) in his review of prenatal loss in a number of species, reported the following estimates: 29.0 percent prenatal loss in the ferret, 20 to 48 percent in sheep, 9.3 percent in the shrew and approximately 30 percent in man. Carr (1977) concluded, that in spite of a large amount of variation, because of experimental methods, in the estimates of pregnancy wastage among various species, mammals rarely lose less than 30 percent of their fertilized ova and that in most species, the total amount of prenatal loss is approximately 40-50 percent.

The validity of estimating prenatal loss by comparing corpora lutea counts with implants has been discussed at some length by Batten and

Berry (1967). In the situation where fewer corpora lutea are found than implants (not seen in the present study), polyovulation, which is estimated to occur about 10.0 percent of the time in primiparous mice (Kent, 1960) and monozygotic twinning, estimated to occur about 1.0 percent of the time in laboratory mice (Wallace and Williams, 1965) have been suggested as explanations. Some situations result in an overestimation of prenatal loss. Occasionally, what appears to be a corpus luteum retains a mature oocyte and therefore prevents its fertilization and implantation (Jones and Krohn, 1961). Some ova are released normally, but are not fertilized. Bowman and Roberts (1958) found 12.6 percent of 452 ova recovered from the fallopian tubes of 41 laboratory mice to be of "normal" appearance, but not yet fertilized.

While the relative importance of these different effects on prenatal mortality cannot be estimated with certainty, it appears reasonable to assume, that the number of corpora lutea counted represents a fairly accurate indication of the number of ova available for fertilization.

Comparison of different populations must be done with reservation because as Batten and Berry (1967) point out, the age of the population should be considered since differences in ovulation rates have been shown to be related to maternal age (Finch, 1978). This problem is probably not too significant in the populations used in this study since Philpott and Petras (unpublished) have found, that the majority of population members are less than one year old and all populations

originate about the same time.

Material and Methods

Source of Animals

Three hundred and forty-seven non-juvenile female mice were obtained from thirteen samples trapped in corn cribs in Essex and Kent counties in south-western Ontario. The samples were taken between June and August of 1977 and in February and April of 1978. Recapture studies show that between 50 and 80 percent of animals residing within cribs are captured (Philpott and Petras, unpublished).

Procedures

The mice were bled from the orbital sinus, into a mammalian saline-sodium citrate solution (5% sodium citrate in 0.8% NaCl). Blood was processed for starch gel electrophoresis according to the procedure described by Biddle and Petras (1967).

Electrophoretic typing of a number of protein systems was conducted. These included esterases controlled by loci Es-2 and Es-3 (Petras, 1963; Petras et al., 1969), phosphoglucomutase (Harris and Hopkinson, 1976), lactate dehydrogenase (Martin and Petras, 1969) glucose phosphate isomerase (Harris and Hopkinson, 1977) and hemoglobin (Petras et al., 1969).

Females of three populations were weighed before being sacrificied by cervical dislocation.

Ovaries and uteri were removed from all females and placed in physiological saline for examination. The following counts were made: number of corpora lutea on each ovary, using a Wilde binocular dissecting microscope with magnification of 32x; and the number of embryos in each uterine horn.

The gestational age and uterine position of all embryos were noted and recorded according to Grüneberg (1943) and Thieler (1972). The criteria for this classification involve morphological developments and embryo size.

Ovaries from two of the populations were fixed after superficial counting of corpora lutea and 10 micron thick serial sections, of the ovaries, were made, mounted and stained with haematoxylin and eosin according to standard histological procedure (Galigher and Kozloff, 1964) and examined using 100x magnification. Ovaries from mice of three inbred strains were similarly prepared for comparison with the wild specimens.

All animals were frozen after dissection. The three previously weighed samples were re-weighed six months after freezing to determine individual female weights after removal of the ovaries and uterus. This second weighing, which was done as an after-thought eliminated any error resulting from the presence of the reproductive tract.

All analyses of data and tests of significance were done on a Monroe 1860 calculator.

Results

Mean Number of Live and Resorbed Embryos

Table 17 shows that sample sizes differed considerably among the different populations, ranging from eight females collected from a crib on the Pinsonault Farm (#6, Concession III) to 146 females from the Pigeon 1977 population. Also presented in Table 17, are summaries of data on the number and percentages of animals pregnant and the females with one or more resorbing embryo. Pregnant females, that is, females with live embryos appear to make up between 50 and 60 percent of the adult female population, of each sample, with an overall mean of 54.5. The number of animals with all embryos dead (100 percent resorbed) is the difference between the number of females with embryos and the number of females with embryos and 100 percent resorbed.

Trapping at various times of the year showed that implantation occurred in all seasons. There appears, however, to be a slight deficiency in the number of females with implants during the winter and early spring. This will be discussed at greater length later.

Table 18 presents the number of females per sample, the number of females excluding those with 100 percent resorbed litters and the number of pregnant females per sample. The mean number and standard deviation of live embryos and the implants for each sampling site are also presented. The mean number of live embryos per pregnant female per sample range from 4.9 to 7.8, while the mean number of implants for the same animals ranged from 5.0 to 7.8. A comparison of the mean

Table 17: Pregnancy data from females of natural populations of Mus musculus

Population	Collection Date	Number of Females	Number of Females with Embryos	Number of Females with Embryos & 100% Resorption	Number of Females with Resorbed Embryos
Caron	9/6/77	12	7 (58.3)*	8 (66.7)	0 (0)
Price, N & S	17/6/77	14	9 (64.3)	9 (64.3)	5 (55.6)
15th Concession	25-26/7/77	14	12 (85.7)	12 (85.7)	5 (41.7)
7th Concession	25/7/77	33	16 (48.5)	24 (72.7)	9 (75.0)
Ouellette	27/7/77	19	11 (57.9)	12 (63.1)	6 (58.3)
Houle	4/8/77	43	22 (51.2)	24 (55.8)	7 (37.5)
Bondy Lt.	9/8/77	10	5 (50.0)	6 (60.0)	0 (16.7)
Pigeon	10/8/77	126	69 (54.8)	87 (69.0)	15 (37.9)
Bondy, SR & NR	15/8/77	8	6 (75.0)	6 (75.0)	3 (50.0)
McKim	24/8/77	28	17 (60.7)	17 (60.7)	6 (35.3)
Pinsonault #6	23/2/78	8	2 (25.0)	3 (37.5)	1 (50.0)
Pinsonault J.R.	28/2/78	15	4 (26.7)	5 (33.3)	0 (0)
Pigeon	8/4/78	17	9 (52.9)	10 (58.8)	1 (20.0)
Pooled		347	189 (54.5)	223 (64.3)	58 (30.7

* Percentage of females

Table 18: Mean number of live and resorbed embryos per females of each population

	All Females Collected			All Females Excluding 100 Percent Resorbed			Pregnant Females		
	Number of Females	Live Embryos	Implanted Embryos	Number of Females	Live Embryos	Implanted Embryos	Number of Females	Live Embryos	Implanted Embryos
Caron	12	4.17 ±3.95*	4.83 ±3.86	11	4.54 ±3.91	4.54 ±3.91	7	7.14 ±1.95	7.14 ±1.95
Price, N & S	14	3.50 ±2.85	4.28 ±3.71	14	3.50 ±2.85	3.50 ±2.85	9	5.44 ±1.13	6.67 ±2.12
15th Concession	14	4.86 ±2.63	5.19 ±2.72	14	4.86 ±2.63	4.86 ±2.63	12	5.67 ±1.28	6.33 ±1.87
7th Concession	33	3.03 ±3.33	5.03 ±3.48	25	4.00 ±3.28	4.60 ±3.82	16	6.25 ±1.48	7.31 ±1.78
Ouellette	19	3.32 ±3.06	4.21 ±3.68	18	3.50 ±3.03	4.17 ±3.78	11	5.73 ±1.27	6.82 ±2.09
Houle	43	2.81 ±2.94	3.42 ±3.20	41	2.95 ±2.94	3.22 ±3.14	22	5.50 ±1.34	6.00 ±1.15
Bondy Lt.	10	2.50 ±2.92	3.00 ±2.87	9	2.78 ±2.95	2.78 ±2.95	5	5.00 ±1.87	5.00 ±1.87
Pigeon	126	2.88 ±2.85	3.79 ±2.80	108	3.36 ±2.81	3.63 ±2.94	69	5.26 ±1.50	5.68 ±1.34
Bondy, SR & NR	8	5.38 ±3.46	5.88 ±3.80	8	5.38 ±3.46	5.38 ±3.46	6	7.17 ±1.17	7.83 ±1.33
McKim	28	2.96 ±2.69	3.48 ±2.90	28	2.96 ±2.69	2.96 ±2.69	17	4.88 ±1.50	5.53 ±1.28
Pinsonault #6	8	1.50 ±2.78	2.50 ±3.32	7	1.71 ±2.91	1.86 ±3.04	2	6.00 ±2.00	6.50 ±1.50
Pinsonault J.R.	15	2.07 ±3.53	2.67 ±3.88	14	2.21 ±3.61	2.21 ±3.61	4	7.75 ±1.64	7.75 ±1.64
Pigeon	7	3.12 ±3.10	3.47 ±3.05	16	3.31 ±3.10	3.38 ±3.12	9	5.89 ±1.37	6.00 ±1.25

* Standard Deviation

number of live embryos and mean number of implants per female in each sample results in a measure of post-implantation mortality. This measure is however, at best, the lower limit of post-implantation mortality because of reasons to be discussed later.

Also presented in Table 18, are estimated of the mean number of offspring to be produced by the total adult female population at the time of sampling. The mean number of live embryos per female for each population, and the mean number of implants per female for each population range from 1.5 to 5.4 and 2.5 to 5.9, respectively. If females with 100 percent resorbed litters are excluded from the calculations, the mean values for these parameters change to ranges of 1.7 to 5.4 and 1.9 to 5.4. This was done in an attempt to eliminate bias resulting from resorption of a litter due to maternal stress encountered in trapping and subsequent handling. Evidence in support of this calculation came from a population captured on June 13th, 1977 but not sacrificed until a week later. Of 29 adult females, 20 (69 percent) were not pregnant at the time of autopsy, 5 (17.2 percent) had totally resorbed litters, and only 4 (13.8 percent) were still pregnant. This problem was minimized in the present study by prompt autopsy of animals after capture. On the basis of these findings, it was decided to exclude females with 100 percent resorbed litters from the summary of data presented for this study.

While examining the differences in the mean number of offspring per female when all females in a sample are considered, a significant correlation ($r = 0.9944$, $n=12$) between the number of pregnant animals

and the total sample size was obtained. As one would expect, more pregnant animals are found in large populations than in small ones, hence, more embryos, but actual changes in percent of pregnant animals is not, however, predictable from sample size.

The mean number of resorbed embryos per pregnant female are presented in Table 19. The range of resorbed embryos per female with implants extends between 0.6 (12%) and 1.8 (23%).

It is interesting to note that the range of mean values for animals with at least one resorption but excluding totally resorbed litters was between 1.3 and 2.2. The mean of this range was 1.8 ± 0.3 which suggests that animals have more than one resorption if they have any at all. If females with at least one resorbed embryo (including those females with totally resorbed litters) are examined, the mean number of resorptions per female was observed to range between 1.6 (32%) and 3.7 (47%).

Differences Between Populations for Reproductive Parameters

To determine if the samples differed from one another for such reproductive parameters as mean number of implants, live and resorbed embryos, one-way analyses of variance were done. Table 20 summarizes the results.

In order to detect sample differences due to seasonal conditions, analyses were performed on samples grouped according to time of trapping. If the samples obtained between June, 1977 and April, 1978 are examined, a significant F value is obtained for the mean number of

Table 19: Mean number of embryos resorbed per female

Sample	Resorbed Per Female with Live Implants	Resorbed Per Female with Live Implants & ≥ one Resorption
Caron	---	---
Price, N & S	1.22 ± 1.48 (9)*	2.20 ± 1.30 (5)
15th Concession	0.67 ± 0.89 (12)	1.60 ± 0.55 (5)
7th Concession	1.06 ± 1.12 (16)	1.70 ± 0.95 (10)
Ouellette	1.09 ± 1.30 (11)	2.00 ± 1.10 (6)
Houle	0.50 ± 0.91 (22)	1.57 ± 0.98 (7)
Bondy Lt.	---	---
Pigeon	0.42 ± 0.98 (69)	1.93 ± 1.22 (15)
Bondy, SR & NR	0.67 ± 0.82 (6)	1.33 ± 0.58 (3)
McKim	0.65 ± 1.06 (17)	1.83 ± 0.98 (6)
Pinsonault #6	0.14 ± 0.38 (7)	---
Pinsonault J.R.	---	---
Pigeon Apr. '78	0.11 ± 0.33 (9)	---

* Number of females

Table 20: One-way analysis of variance of pregnancy parameters and season of trapping

Parameter Analysed	Samples Included In Analysis	
	Summer Populations (June '77 - Aug. '77)	All Populations (June '77 - Apr. '78)
$\bar{X}$ No. of Implants Per Female with Live Implants	$F = 3.400$ * $p \leq 0.025$ $df = 8,196$	$F = 3.200$ ** $p \leq 0.01$ $df = 11,210$
$\bar{X}$ No. of Live Embryos Per Female with Live Embryos	$F = 2.406$ NS $p \geq 0.05$ $df = 8,165$	$F = 2.496$ * $p \leq 0.025$ $df = 11,177$
$\bar{X}$ No. of Resorbed Embryos Per Female with Some Live Embryos	$F = 1.841$ NS $p \geq 0.10$ $df = 8,198$	$F = 1.456$ NS $p \geq 0.10$ $df = 11,212$

* Significant at 2.5% level
** Significant at 1% level

implants and the mean number of live embryos. Mean numbers of resorbed per population did not differ significantly among the samples.

If samples obtained only between June and August, that is, in the summer of 1977, are examined, a significant F value is obtained for the mean number of implants per female, but not, for the mean number of live or resorbed embryos.

Significant F values for some parameters indicate that some population samples differ significantly from others.

The Student-Newman-Keuls Test was employed to identify different populations (Sokal and Rohlf, 1969). For mean number of implants per female or the summer populations no sample is observed to differ significantly from any other population with the SNK test.

In the analyses of all of the samples for mean number of implants per female only the summer sample trapped at Pigeon's farm differs significantly from the population trapped at Pinsonault, Jacob Road (Table 21). No significant differences were observed in the mean number of implants per female of the summer samples.

Estimates of Corpora Lutea Numbers

For the case of mean number of live embryos per female for all populations, the sample from McKim's farm differs significantly from that obtained at Caron's farm and also at Pinsonault, Jacob Road. Pigeon's sample trapped in August, 1977 also differs significantly from

Table 21: Student-Newman-Keuls Test for pregnancy parameters of populations captured in June - August 1977, and June - April 1978. Any sample not underlined differs from the first population of that comparison at the 5% level of significance

$\bar{X}$ Implants / Female - Summer Populations F = 3.4003

McKim	Pigeon	Houle	15th Conc.	Bondy	Ouellette	Price	7th Conc.	Caron
1	2	3	4	5	6	7	8	9

$\bar{X}$ Implants / Female - All Populations F = 3.2003

McKim	Pigeon Apr.	Pigeon	Houle	15th Conc.	Bondy	Pin.#6	Price	Ouellette	7th Conc.	Caron	Pin. J.R.
1	2	3	4	5	6	7	8	9	10	11	12

$\bar{X}$ Live / Female - All Populations F = 2.4961

McKim	Pigeon	Price	Houle	15th Conc.	Ouellette	Pigeon Apr.	Pin.#6	Bondy	7th Conc.	Caron	Pin. J.R.
1	2	3	4	5	6	7	8	9	10	11	12

the sample obtained from Pinsonault, Jacob Road. These results shall be discussed later.

It should be noted, that due to insensitivity in the SNK test slight differences between samples may not be recognized as significant, in spite of the ANOVA results.

Correlation Between Pregnancy Parameters

Relationships among a number of pregnancy parameters were investigated utilizing correlation analysis. Since the populations did not differ significantly for these parameters, they were combined. For the mean number of implants versus resorbed embryos, a correlation coefficient of 0.9417 ($p \leq 0.01$) was obtained (Sokal and Rohlf, 1969).

The correlation coefficients are similar ($r = 0.9206$, $p \leq 0.01$ - and $r = 0.8972$, $p \leq 0.01$) when the number of live embryos is compared to the number of resorbed embryos. Therefore, as the number of implants increases per female, there is a direct increase in both the number of live and number of resorbed embryos.

Estimates of Corpora Lutea Numbers

Estimates of corpora lutea numbers may be utilized in determining estimates of pre- and post-implantation mortality because the number of corpora lutea approximates the number of ova available for fertilization (Batten and Berry, 1967).

Frequently, the number of corpora lutea of an ovary are determined macroscopically. This procedure reportedly results in an underestimate

of number of corpora lutea present (Brambell, 1948). As a result, a comparison was made between macroscopic determination of the number of corpora lutea and counts from histological preparations in several inbred mouse strains and a number of wild-caught mice. The results are presented in Table 22..

Comparison of the macro and micro methods shows a difference of ± 1 corpus luteum per ovary. In an ovary with few corpora lutea this represented a difference as high as 18 percent. These differences appear larger than the 4.5 percent difference reported by Brambell (1948) in rabbits and suggest that values for corpora lutea counts may be underestimated. Rabbits however may be more amenable for this type of examination than mice.

Differences were found among the number of corpora luteal counts of inbred strains and the inbreds pooled have greater numbers than the wild-caught sample. In order to determine if any of the inbred strains or wild-mice differed from one another or differed because of the corpora lutea counting methods a one-way analysis of variance and a generalized ANOVA were carried out. No statistically significant difference among counts for the inbred and wild-mice and between the two counting methods was found.

Similarly, a chi-square test showed the corpora lutea estimates obtained by the two techniques not to be significantly different.

Table 22: Mean number of corpora lutea per ovary (inbred and wild mice) as determined by macro and micro counting techniques on the same individuals

Strain	Corpora Lutea (Macro)	Corpora Lutea (Micro)	Percent Difference Macro VS Micro
C57BL/6J	5.18 ± 2.43* (N = 50)	5.36 ± 2.32 (N = 50)	3.4 Under Estimate
DBA	6.00 ± 2.00 (N = 12)	7.08 ± 2.06 (N = 12)	18.0 Under Estimate
CBA	5.38 ± 2.00 (N = 8)	5.25 ± 1.98 (N = 8)	2.3 Over Estimate
Pooled Inbred	5.34 ± 2.30 (N = 70)	5.70 ± 2.29 (N = 70)	6.7 Under Estimate
Wild	4.00 ± 1.97 (N = 22)	4.72 ± 2.02 (N = 22)	18.0 Under Estimate

Generalized ANOVA

Number of Corpora Lutea as Determined by Macro and Micro Counting Methods (Inbred and Wild Mice)

Source	SS	df	MS	F	
Between Macro & Micro	7.4402	1	7.4402	0.5520	$p \geq 0.05$
Between Strains	80.8686	6	13.4781	2.7343	$p \geq 0.05$
Subtotals	88.3088	7	---		
Between Determinations Within Strains	867.5553	176	4.9292		
Totals	955.8641	183	---		

* Standard Deviation

Variability in Mean Corpora Lutea Numbers

To determine whether differences in mean corpora lutea number among the populations are significant, an analysis of variance was performed.

The analysis was performed both including and excluding females with totally resorbed litters. Significant F values were obtained for both sets of data (Table 23). Further analysis using Student-Newman-Keuls Tests (SNK) revealed which populations differed significantly.

Pre- and Post-Implantation Mortality

Estimates of pre- and post-implantation mortality within the samples are presented in Table 24. In all cases, the mean number of corpora lutea was greater than the mean number of implants. The mean number of corpora lutea ranged from 5.8 to 13.5 for pregnant females when totally resorbed litters were excluded. The mean pre-implantation loss per pregnant female, per sample, was determined by subtracting the mean number of implants per pregnant female from the mean number of corpora lutea counted per pregnant female in each sample. The range for the sample means was between 0.44 and 5.75 ova lost per pregnant female. This represents a loss of between 5.7 percent and 42.6 percent for females with implants. Differences in sample sizes may in part be responsible for the broad range.

Total pre-natal loss per female was determined using the following formula from Batten and Berry (1967):

Table 23: Analysis of variance of mean number of corpora lutea per female resulted in significant F values. Student-Newman-Keuls Test utilizes stepwise comparisons of the ranked mean values. The first sample population mean in each comparison is compared to all means smaller than the chosen first mean. Any sample not underlined differs from the first mean of that comparison at the 5 percent level of significance.

Anova - Mean Number of Corpora Lutea/Pregnant Females

df = 10,165
F = 8.0170, $p \leq 0.001$

Student-Newman-Keuls Test (1 percent level of Significance)

1	2	3	4	5	6	7	8	9	10	11
Bondy Lt.	Pigeon	McKim 15 Conc.	Houle 7 Conc.	Pin. #6	Ouellette	Pigeon (Apr.)	Bondy, SR	Pin. NR	J.R.	

Table 24: Mean number of live embryos, mean number of implants and mean number of corpora lutea per female. Estimates of pre-implantation and prenatal embryonic loss

Population	Live Per Female with Live Implants	Corpora Lutea Per Female with Live Implants	Pre-Implantation Loss Per Female (Excl. 100% Rd.)	Prenatal Loss Per Female (Excl. 100% Rd.)
Caron	7.14 ± 1.95 (7)*	---	---	---
Price, N & S	5.44 ± 1.13 (9)	---	---	---
15th Concession	5.67 ± 1.78 (12)	---	---	---
7th Concession	6.25 ± 1.48 (16)	7.75 ± 3.24 (16)	0.44 (5.7%)	19.4%
Ouellette	5.73 ± 1.27 (11)	8.82 ± 3.52 (11)	2.00 (24.4%)	35.0%
Houle	5.50 ± 1.34 (22)	6.43 ± 1.08 (22)	0.43 (6.7%)	14.5%
Bondy Lt.	5.00 ± 1.87 (5)	5.80 ± 2.49 (5)	0.80 (13.8%)	13.8%
Pigeon	5.26 ± 1.50 (69)	6.28 ± 1.59 (69)	0.60 (9.5%)	16.2%
Bondy, SR & NR	7.17 ± 1.17 (6)	---	---	---
McKim	4.88 ± 1.50 (17)	---	---	---
Pinsonault #6	6.00 ± 2.00 (2)	8.00 ± 2.00 (2)	1.50 (18.8%)	25.0%
Pinsonault J.R.	7.75 ± 1.64 (4)	13.50 ± 4.03 (4)	5.75 (42.6%)	42.6%
Pigeon - Apr.	5.89 ± 1.37 (9)	8.89 ± 1.91 (9)	2.89 (32.5%)	33.7%

* Number of Females

$$\text{Percent loss} = 1 - \frac{\text{live implants/(pregnant female)}}{\text{mean number of corpora lutea}}$$

The range of pre-natal loss estimates was 13.8 to 42.6 percent for all females with implants. Estimates of pre-natal loss when totally resorbed litters are included in the calculations are presented in Table 38, Appendix B.

Mortality and Gestation Period

Litters were grouped into those appearing less than 10 days gestational age and those greater (Table 25). The ten day period was chosen since it is approximately the mid-point of gestation in the mouse, and since this is also an important time in the developmental sense, with many morphological changes occurring. For instance, limb buds and facial structures are commencing to form at this time (Thieler, 1972).

Overall, the litters observed, were distributed uniformly in the two gestational periods. This indicates little or no synchronization of ovulation in natural populations. The majority of litters contain no resorptions.

The data suggest that there is a greater likelihood of total litter resorption in litters of less than 10 days gestation (26.5%) than in older litters (3.6%).

Table 25: Number of litters of less than or greater than 10 days of gestation and numbers of litters with no, some, and 100 percent resorption

Sample	Litters ≤ Day 10	0 Rd.	Litters ≥ 1 Rd.	100% Rd.	Litters > Day 10	0 Rd.	Litters ≥ 1 Rd.	100% Rd.
Caron	7 (87.5%)	6 (85.7%)	0	1 (14.3%)	1 (12.5%)	1 (100%)	0	0
Price, N & S	3 (33.3%)	0	3 (100%)	0	6 (66.7%)	4 (66.7%)	2 (33.3%)	0
15th Concession	3 (25.0%)	2 (66.7%)	1 (33.3%)	0	9 (75.0%)	5 (55.6%)	4 (44.4%)	0
7th Concession	10 (41.7%)	1 (10.0%)	1 (10.0%)	8 (80.0%)	14 (58.3%)	6 (42.8%)	8 (57.1%)	0
Ouellette	7 (58.3%)	4 (57.1%)	2 (28.6%)	1 (14.3%)	5 (41.7%)	1 (20.0%)	4 (80.0%)	0
Houle	6 (25.0%)	4 (66.7%)	1 (16.7%)	1 (16.7%)	18 (75.0%)	11 (61.1%)	6 (33.3%)	1 (5.6%)
Bondy Lt.	4 (66.7%)	3 (85.0%)	0	1 (25.0%)	2 (33.3%)	2 (100%)	0	0
Pigeon	49 (56.3%)	33 (67.3%)	0	16 (32.6%)	38 (43.7%)	21 (55.3%)	15 (34.5%)	2 (5.3%)
Bondy, SR & NR	4 (66.7%)	3 (75.0%)	1 (25.0%)	0	2 (33.3%)	0	2 (100%)	0
McKim	7 (41.2%)	7 (100%)	0	0	10 (58.8%)	4 (40.0%)	6 (60.0%)	0
Pinsonault #6	2 (66.7%)	1 (50.0%)	0	1 (50.0%)	1 (33.3%)	0	1 (100%)	0
Pinsonault J.R.	4 (80.0%)	3 (75.0%)	0	1 (25.0%)	1 (20.0%)	1 (100%)	0	0
Pigeon - Apr.	7 (70.0%)	7 (100%)	0	0	3 (30.0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Totals	113 (50.7%)	74 (65.5%)	9 (8.0%)	30 (26.5%)	110 (49.3%)	57 (51.8%)	49 (44.5%)	4 (3.6%)

Effects of Uterine Horns on Embryo Mortality

At the time of autopsy of the females, the total number of embryos, both alive, dead and resorbed, were recorded for each uterine horn. Analysis (χ^2) was carried out for each population sample individually and for the samples pooled, to determine if the two horns differed significantly. The results, summarized in Table 26, indicate there were no significant differences between the two uterine horns in individual samples (except that from 15th Concession), and in the pooled data. More litters with one or more, but not totally resorbed, embryos existed in the group of litters of greater than 10 days gestation. No significant difference existed in the number of litters with no resorptions between the two time groups.

Effect of Implantation Position

At the time of autopsy, implant sites were numbered sequentially beginning at the ovarian end in each horn. A summary of the number of implants and resorptions is presented in Table 27. Due to the small sample sizes in some cases, left and right uterine horns were combined. The ratio of resorbed embryos to implants is also given for each uterine position. Totally resorbed litters were included in this table to provide total mortality per uterine site.

Due to the numbering system, there are fewer embryos at the sites closest to the cervix. The percent of embryos resorbed differs for each position and the position with the greatest percent varies from sample to sample. No one uterine position therefore, appears

Table 26: Contingency chi-square analysis of live embryos and resorptions in left and right uterine horns

Sample	Left		Right		X^2
	No. Live	No. Rd.	No. Live	No. Rd.	
Caron	25	4	25	4	0.00
Price, N & S	27	5	22	6	0.33
15th Concession	45	2	23	6	5.14*
7th Concession	58	29	42	36	2.83
Ouellette	29	9	34	8	0.25
Houle	60	15	54	11	0.21
Bondy Lt.	13	2	12	3	0.24
Pigeon	162	53	202	67	0.004
Bondy, SR & NR	21	1	22	3	0.83
McKim	34	3	48	8	0.81
Pinsonault #6	7	0	5	1	1.26
Pinsonault J.R.	18	6	13	3	0.21
Pigeon	27	3	26	3	0.001
Pooled Samples	526	132	528	159	1.88

* Significant at 5% level

Table 27: Number of implanted and resorbed embryos per relative site within the uterus with left and right horns combined.

	Caron		7th Conc.		15th Conc.		Ouellette		Houle	
Uterine Position										
1	2/16*	(12.5)**	20/47	(42.5)	3/23	(13.0)	5/24	(20.8)	9/47	(19.1)
2	2/15	(13.3)	18/43	(41.8)	4/20	(20.0)	5/22	(22.7)	7/43	(16.2)
3	2/12	(16.7)	14/35	(40.0)	1/12	(8.3)	4/16	(25.0)	7/32	(15.6)
4	2/10	(20.0)	12/24	(50.0)	0/8	(00.0)	2/8	(25.0)	2/19	(10.5)
5	0/4	(00.0)	2/11	(18.2)	0/6	(00.0)	0/4	(00.0)	0/5	(00.0)
6	0/1	(00.0)	1/3	(33.3)	0/4	(00.0)	1/4	(25.0)	0/1	(00.0)
7			0/1	(00.0)	0/3	(00.0)	0/2	(00.0)		
Total	8/58	(13.8)	67/164	(41.1)	8/76	(10.5)	17/80	(21.2)	23/147	(15.6)
100% Rd.	8/0	(00.0)	49/0	(00.0)	0/0	(00/0)	5/0	(00.0)	15/0	(00.0)
Total	0/50	(00.0)	18/115	(15.8)	8/76	(10.5)	12/75	(16.0)	8/132	(6.1)

Continued...

* Resorption/Implantation

** Percent Resorption

Table 27: Continued

Bondy L.T.		Pigeon		Bondy		McKim		Pinson. #6		Pinson. J.R.		Pigeon Apr. '78	
2/11	(18.2)	43/165	(26.1)	1/12	(8.3)	3/34	(8.8)	2/6	(20.0)	2/10	(20.0)	2/18	(11.1)
2/11	(18.2)	41/150	(27.3)	1/11	(9.1)	5/29	(17.2)	3/6	(50.0)	2/9	(22.2)	3/18	(16.7)
1/7	(14.3)	23/97	(23.7)	0/10	(00 0)	1/17	(5.9)	2/5	(40 0)	2/9	(22.2)	1/13	(7.7)
0/1	(16.0)	8/50	(16.0)	2/8	(25.0)	1/10	(10.0)	1/2	(50.0)	1/6	(16.7)	0/7	(00.0)
		3/17	(17.6)	0/4	(00.0)	1/3	(33.3)	0/1	(00.0)	1/4	(25.0)	0/3	(00.0)
		2/4	(50.0)	2/2	(00.0)					1/2	(50.0)		
		0/1	(00.0)										
5/30	(16.7)	120/484	(24.8)	4/47	(8.5)	11/93	(11.8)	8/20	(40.0)	9/40	(22.5)	6/59	(10.2)
5/0	(00.0)	85/0	(00.0)	0/0	(00.0)	0/0	(00.0)	7/0	(00.0)	0/0	(00.0)	5/0	(00.0)
0/25	(00.0)	35/399	(8.8)	4/47	(8.5)	11/93	(11.8)	1/13	(7.7)	9/40	(22.5)	1/54	(1.8)

particularly vulnerable to embryo mortality. There also appears no overall set pattern for resorption in the various samples.

The resorbed to implanted embryo ratios range from 0.088 to 0.411 per position for the sample totals.

Exclusion of totally resorbed litters, because of possible bias, did not alter the conclusions.

Relationships Between Female Weight and Pregnancy Parameters

Females of three populations were weighed before and after autopsy. Mean sample weights along with standard deviation and sample size, are presented in Table 28. Mean weights of pregnant animals were, as would be expected, higher than population means, except for the Bondy sample. Even after removal of ovaries and the uterus, pregnant animals had a higher mean weight in the Pigeon and Houle populations. From the weights of non-pregnant animals before and after autopsy, it was estimated, that the ovaries and uterus which were removed from each animal weighed about 1.0 gram.

One-way ANOVA revealed no significant differences among the weights after autopsy of females from various populations ($F = 2.835$, d.f. = 2,176, $p \geq 0.25$).

Correlation of Female Body Weight and Pregnancy Parameters

Potential correlations between female weights and various pregnancy parameters were also investigated using correlation analysis. Female weights both before and after autopsy were included

Table 28: Mean weight of females from populations trapped at the Pigeon, Houle and Bondy farms

Parameters	Sample Source		
	Pigeon	Houle	Bondy Lt.
Mean Weight of Total Female Population	19.89 ± 4.23* (126)**	18.86 ± 4.45 (43)	20.08 ± 4.44 (10)
Mean Weight of Total Female Population After Autopsy	17.14 ± 3.10 (126)	15.85 ± 3.31 (43)	17.41 ± 2.43 (10)
Mean Weight of Females with Live Embryos	20.94 ± 3.97 (87)	20.73 ± 3.77 (24)	19.59 ± 5.70 (6)
Mean Weight of Females with Live Embryos After Autopsy	17.75 ± 2.67 (87)	16.21 ± 2.50 (24)	16.34 ± 2.07 (6)

* Standard deviation
** Number of females

(Table 29) for three populations.

Significant correlation coefficients, were obtained for most analyses. Only in the cases of numbers of resorbed versus weight before autopsy for the Houle sample, and live and implant numbers versus weight after autopsy for the Bondy sample, were non-significant values obtained.

In the Pigeon sample, before and after autopsy, heavier females had a larger number of implants and live embryos than did lighter females. Both before and after autopsy, lighter animals had more resorbed embryos than heavier animals. The same results were obtained for the Houle sample and for implants and number of live embryos for the Bondy sample.

It is expected, that before autopsy, female weight will be correlated with number of implants, because litters will increase females' weights. After autopsy, when all embryonic and reproductive tissue is removed, heavier and perhaps therefore, larger animals still show the tendency to have larger litters.

Correlation Between Pregnancy Parameters and Season of Trapping

Correlations were also sought between several pregnancy parameters and season of trapping (Table 30). For this, each population was assigned a number based on the number of days which had elapsed from the first day of trapping, June 6, 1977.

First, populations trapped between June and August, 1977 were compared for mean number of implants per sample versus period of

Table 29: Correlation of pregnancy data with female weight

Correlation		Pigeon	Houle	Bondy Lt.
Impl. VS Weight	r	0.227 **	0.360 **	0.475 **
	t	2.153	0.807	1.080
	n	87	24	6
Live VS Weight[x]	r	0.212 **	0.475 **	0.481 *
	t	1.774	2.413	0.949
	n	69	22	5
Rd. VS Weight[xx] (Excl. 100% Rd.)	r	-0.759 **	-0.557 **	---[xxx]
	t	-4.203	-1.499	
	n	15	7	
Impl. VS Weight	r	0.227 **	0.484 **	0.304 NS
	t	2.140	2.596	0.637
	n	87	24	6
Live VS Weight	r	0.228 **	0.468 **	0.306 NS
	t	1.872	2.371	0.556
	n	69	22	5
Rd. VS Weight (Wt. after Aut.) (Excl. 100% Rd.)	r	-0.635 **	-0.341 *	---
	t	-2.966	-0.811	
	n	15	7	

x Animals with 100% Rd. litters excluded
xx Animals with no Rd. excluded
xxx Only 1 Data Point - 100% Rd.

* Significance at 5% level
** Significance at 1% level

Table 30: Correlation of pregnancy parameters with season of trapping*

Time Period	Mean No. Live Implants Per Female With Live Implants	Mean No. Implants Per Female With Live Implants	Mean No. Resorbed Per Female With Live Implants
June '77 - Aug. '77	r -0.388 **	-0.395 **	0.003 NS
	t -1.189	-1.215	0.008
	N 10	10	10
Aug. '77 - Apr. '78	r -0.645 NS	-0.455 NS	-0.684 NS
	t -0.843	-0.512	-0.937
	N 3	33	3
June '77 - Apr. '78	r 0.060 NS	0.273 **	-0.432 **
	t 0.198	0.942	-1.590
	N 13	13	13

* Counting of Time, June 9, 1977 = Day 1

** Significance at 1% Level

Continued...

Table 30: Continued

Time Period	No. Live Implants Per Adult Female Population	No. Implants Per Adult Female Population	No. Resorbed Per Adult Female Population
June '77 - Aug. '77	-0.395 ** -1.215 10	-0.388 ** -1.189 10	-0.163 NS -0.467 10
Aug. '77 - Apr. '78	0.969 ** 3.937 3	-0.334 ** -1.225 3	-0.149 NS -0.150 3
June '77 - Apr. '78	-0.232 ** -0.825 13	-0.615 ** 2.587 13	-0.342 ** -1.208 13

trapping and mean number of live implants versus time of trapping. Both analyses gave significant, negative correlation coefficients.

The same statistical analysis was applied to the populations trapped during the second trapping period extending from August, 1977 to April, 1978. Significant correlations were observed only when the mean number of live implants per total adult female sample (pregnant and non-pregnant females) and the mean number of implants per total adult female sample were compared with time. Since the former coefficient is positive, and the latter negative, it appears that the number of live implants increases while the total number of implants for all females decreases, that is, there is a decrease in embryo mortality later in the fall and winter trapping period. There was no significant correlation between mean number of resorbed embryos and time.

When these data from the fall and winter period are compared with those from the summer trapping period, June, 1977 to August, 1977, different trends are observed. For the summer trapping period, both the mean number of live and mean number of implanted embryos appear to decrease per populations' pregnant females and total female sample component, as the summer trapping season progresses.

When both the summer and winter trapping periods are considered together as a single June to April period, a different pattern of significant correlations is again obtained. All parameters except mean number of live implants per pregnant female versus time are significantly correlated, and only mean number of implants per pregnant

female versus time exhibits a positive correlation. Only for this series of analyses were significant correlations of mean numbers of resorbed embryos and time observed.

These results indicate, that the later in the period that trapping is conducted, the more implants there were per pregnant females. Since there were fewer females pregnant however, there was a net decrease in the total number of implants, live and resorbed embryos during the winter and early spring.

Further discussion of these results is presented later.

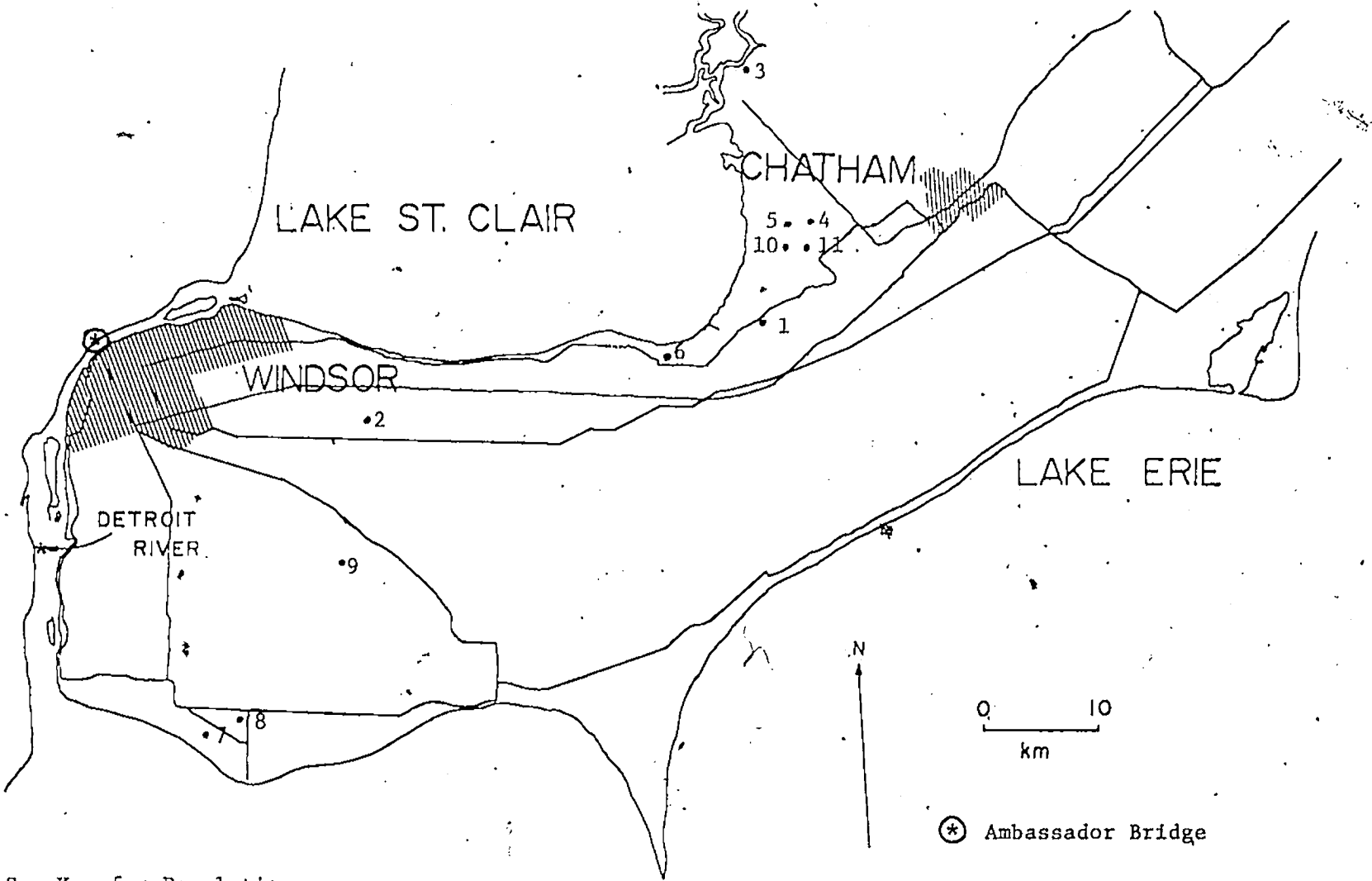
Association of Pregnancy Parameters and Geography

Figure 2 shows the location of trapping sites and their distance from the western end of Windsor, Ontario which is situated near the source of heavy industrial air pollution on the west bank of the Detroit River.

Correlation analysis relating distance of trapping sites from Windsor (Table 31) indicates, that for the sample consisting of pregnant females there is a significant positive correlation ($r = 0.4860$, $r = 0.4648$) between the numbers of live and implanted embryos respectively, and the distance of the trapping site from Windsor. There is no significant correlation between the number of resorbed embryos per pregnant female and the distance of the trapping site from Windsor. There is a significant negative correlation ($r = -0.2141$) between the number of pregnant animals per trapping site, and distance of the trapping site from Windsor.

Key for Figure 2: Map reference numbers for corn crib population sampling sites

Map Reference Number	Sampling Site	Distance (km.) of Trapping Sites From Ambassador Bridge
1	Caron	56
2	Price, N & S	19
3	15th Concession	58
4	7th Concession	61
5	Ouellette	58
6	Houle	48
7	Bondy Lt., Bondy SR & NR	32
8	Pigeon	32
9	McKim	24
10	Pinsonault #6	58
11	Pinsonault J.R.	60



See Key. for Population Reference Numbers

Table 31: Correlation of pregnancy data with geography

Correlation with Parameters from Females with Live Implants and Distance	Mean Live/Pregnant Female VS Distance	Mean Implants/Pregnant Female VS Distance	Mean Rd./Pregnant Female VS Distance
	r 0.486 **	0.465 **	-0.049 NS
	t 1.844	1.741	-0.162
	N 13	13	13

Correlation with Parameters from Adult Female Population & Distance	Mean Live/Female Pop. VS Distance	Mean Implants/Female Pop. VS Distance	Mean Rd./Female Pop. VS Distance
	r -0.129 NS	0.017 NS	0.470 **
	t -0.432	0.058	1.768
	N 13	13	13

** $p \leq 0.01$

However, if the total adult female population (pregnant and non-pregnant females) per sample is used as the basis for analysis, the only significant correlation obtained is one between mean number of resorbed embryos per population and distance of the trapping site from Windsor ($r = 0.4703$).

These apparently contradictory results shall be discussed later.

In order to more clearly determine if the summer samples differ from one another in terms of their geographic location, two by two contingency chi-square analysis was performed utilizing mean values of live and resorbed embryos, and mean numbers of implants and resorbed embryos. Comparisons were made according to sample location in Essex and Kent Counties in Ontario. Stepwise sequential comparisons were made until each sample had been included in the geographically oriented analyses (Table 32). In no case, within each of the two counties, or in comparison between the two counties, was a significant chi-square value obtained.

In terms of mean numbers of live and resorbed embryos, and mean numbers of implants per sample, there is no significant difference that may be detected by this sort of analysis between any of the samples.

Association of Female Genotype and Pregnancy Parameters

Each female, used in this study, was bled before autopsy, and the blood was processed for starch-gel electrophoretic typing of a variety of protein systems.

Classification of female genotype for each system examined, was

Table 32: Two by two contingency chi-square analysis of sampling sites according to geographic location

Essex County			Live Rd.	Implants Rd.
(1) Bondy Lt. Bondy, SR & NR Pigeon	VS	McKim	$\chi^2 = 0.237$ $p \geq 0.50$	$\chi^2 = 0.320$ $p \geq 0.50$
(2) Bondy Lt. Bondy, SR & NR Pigeon McKim	VS	Price	$\chi^2 = 0.150$ $p \geq 0.50$	$\chi^2 = 0.284$ $p \geq 0.50$
(3) Bondy Lt. Bondy, SR & NR Pigeon McKim Price	VS	Houle	$\chi^2 = 0.013$ $p \geq 0.90$	$\chi^2 = 0.022$ $p \geq 0.50$
Kent County				
(4) Caron	VS	Ouellette 7th Concession	$\chi^2 = 0.700$ $p \geq 0.10$	$\chi^2 = 0.963$ $p \geq 0.10$
(5) Caron Ouellette 7th Concession	VS	15th Concession	$\chi^2 = 0.380$ $p \geq 0.50$	$\chi^2 = 0.406$ $p \geq 0.51$
Essex and Kent Counties				
Caron Ouellette 7th Concession 15th Concession	VS	Bondy Lt. Bondy, SR & NR Pigeon McKim Price Houle	$\chi^2 = 0.015$ $p \geq 0.90$	$\chi^2 = 0.024$ $p \geq 0.50$

compared with pregnancy data per female. Potential patterns of pregnancy data such as a high number of resorbed embryos per female of a particular genotype were examined, but none were found (Table 33).

Discussion

Embryonic mortality estimates for the house mouse populations from Ontario examined in this study range from 1.3 percent to 37.7 percent (mean = 18.2 ± 12.7) for pre-implantation loss, 0.0 percent to 18.3 percent (mean = 8.1 ± 6.2) for post-implantation loss and 13.8 to 42.6 percent (mean = 24.0 ± 10.0) for total prenatal loss.

The post-implantation mortality estimates obtained in this study are in the lower part of the range because, a) litters which have been totally resorbed were excluded, since stress during and after trapping contributed, at least in some cases, to total resorption of a litter and b) in some cases, embryonic mortality might have normally occurred but for the fact the dam was sacrificed and so the litter did not reach full term.

Southern and Laurie (1946) report that a decrease in the number of pregnant animals outside corn ricks was found during the fall months, but that breeding did occur during the winter. The authors also reported, that the same patterns of mating occurred in the ricks during the winter in the U.S.S.R.. Therefore, with suitable protection, mice will breed during cold months, but a lack of adequate cover will probably result in decreased reproductive activity.

Table 33: Association between specific phenotypes at the Hbb and Gpi-1 loci and the reproductive state of 302 females

Phenotypes	Number of Pregnant Animals	Number of Non-Pregnant Animals	Number of 100% Resorbed Litters	Total per Genotype (Frequency)
Hbb^s/Hbb^s				
observed	106	67	17	185
*expected	100.8	66.0	18.1	(0.61)
χ^2	0.268	0.015	0.067	
Hbb^s/Hbb^f				
observed	52	35	13	100
expected	52	35	13	100
χ^2	54.4	35.7	9.8	(0.33)
	0.115	0.014	1.045	
Hbb^f/Hbb^f				
observed	11	5	1	17
expected	9.3	6.1	1.7	(0.05)
χ^2	0.311	0.198	0.288	
$Gpi\text{-}1^a/Gpi\text{-}1^a$				
observed	73	38	14	125
expected	68.1	44.6	12.2	(0.41)
χ^2	0.352	0.977	0.266	
$Gpi\text{-}1^a/Gpi\text{-}1^b$				
observed	52	42	9	108
expected	58.9	38.6	10.6	(0.36)
χ^2	0.613	0.299	0.242	
$Gpi\text{-}1^b/Gpi\text{-}1^b$				
observed	39	21	9	69
expected	37.6	24.6	6.8	(0.23)
χ^2	0.052	0.527	0.712	
Hbb^s/Hbb^f $Gpi\text{-}1^a/Gpi\text{-}1^b$**				
observed	19	15	5	39
expected	21.2	13.9	3.8	(0.13)
χ^2	0.228	0.079	0.379	

* Expected proportions calculated using values from Table 1. (Pregnant = 54.5%; Non-Pregnant = 35.7%; 100% Resorbed = 9.8%)

** Heterozygous at both Hbb and Gpi-1 loci

Biggers et al. (1958) stated that pregnant mice kept in a cold room (5°C) had a significant increase in prenatal mortality, as compared to animals housed in warm (21°C) and hot (28°C) rooms.

The apparent discrepancies between Laurie's findings and those of the present study, may be due to differences in the severity of the winters in the two study sites. The more stringent winter in Ontario may cause more stress than in England, or for that matter the artificial environment utilized by Biggers et al.

Besides seasonal or climatic stress, animals are subjected daily to other environmental factors such as inter and intra-specific interactions which may also have an important influence on prenatal mortality. For example, the sample trapped at Ouellette's farm during the summer of 1977 had a prenatal mortality higher than that of the remaining summer populations and approached that of the winter populations. At least seven rats were observed in the crib. These animals probably resided in and around the corn crib and had a detrimental effect on the mouse population since there was evidence of rat-inflicted injuries on the mice. Similar examples of inter-specific interaction may be shown to occur in winter samples.

Batten and Berry (1967) report that crowding or exposure to strange males may cause an increase in prenatal mortality, due to stress. Hawkeswood (1975) showed that crowding stress can decrease the number of males present in a corn crib, a fact which may influence the rate of migration of strange males into a crib. In the present study,

of 232 animals trapped at the Pigeon farm only 78 were males. This population, the largest of those trapped, and the deficiency of males ($\chi^2 = 24.9$, $p \leq 0.01$) indicates that some competition for space did occur.

Two factors, therefore, climatic condition and inter-intra-specific interaction, may cause stress at sufficiently high enough levels to induce a large amount of prenatal mortality in the mice. A combination of environmental and non-climatic stress factors likely determines the number of females which become pregnant during the winter and also the degree of prenatal mortality which occurs throughout the year. Changes in conditions from year to year in different corn cribs could, in part, be responsible for the variability observed in the size of summer population. To a large extent, the size of a population and its rate of growth is dependent upon founder population size and reproductive activity within the population.

Although biases due to sampling errors and small sample sizes could affect the differences in pregnancy parameters observed between populations, the problem appears to be minimal in this study however, because most parameters do not significantly differ among the populations. Very slight differences in pregnancy parameters however could possibly exist, but not be detected due to small sample size.

Embryonic Development Within the Uterus

Resorptions, in populations sampled for the present study, are spread throughout the uteri, the pattern of resorption being different

in each population, indicating, that no uterine site is particularly vulnerable to embryonic mortality.

Laurie (1946) states that in wild-caught mice from several different types of environment, there was a statistically significant larger number of embryos in the right uterine born than in the left. The findings of Danforth and de Aerle (1928) indicated that there was no difference between the horns of uteri in laboratory mice in terms of embryo numbers. The present study, except for one sample (15th Concession) supports the latter.

The present study has shown that while there may be differences between the counts of corpora lutea per ovary obtained with the macroscopic and histological methods, these two methods do not produce results which differ significantly from one another.

While Brambell (1948) showed that in rabbits, macroscopic counts of corpora lutea on ovaries were about 4.5 percent less than counts obtained microscopically using serial sections of the ovaries. It is difficult to determine whether the differences observed were significant since no statistical analysis was presented.

As described earlier, trapping stress may contribute to increased prenatal mortality in mice. Other forms of stress due to seasonal conditions, and inter/intra-specific interaction may also be involved in determining amounts of pre-natal mortality. In this study, Student-Newman-Keuls tests revealed several differences among populations for such pregnancy parameters as mean numbers of implanted, live and resorbed embryos. Maternal stress may function here also, in

the determination of prenatal mortality. The sample obtained during the winter at the Pinsonault, Jacob Road trapping site had a significantly larger amount of prenatal mortality than populations obtained in the summer. This particular population had a higher mean number of corpora lutea per female than did populations from the summer months. Laurie (1946) examined reproductive patterns in Mus musculus populations from several environments including corn ricks in southern England. These studies led to the conclusion, that wild mice bred throughout the year, and that there was no seasonal difference in either the percentage number of adult females pregnant or the number of embryos per litter.

Variation of Female Weights Among Populations and Correlation of Female Weight and Litter Size

Statistical analysis revealed no significant differences among females' weights in different populations both before and after considering certain errors. Slight underestimation of female weight after autopsy could result from the removal of female reproductive organs (approximately 1 gram) and due to lyophilization of those animals which were frozen.

Laurie (1946) stated that there was no correlation between litter size and female weight. In the British populations studied by Batten and Berry (1967) however, significant correlation between number of implants and maternal weight existed. The findings of the present study agree with Batten and Berry.

Correlation of Pregnancy Data with Trapping Period and Geographic Location

Correlation analysis of pregnancy parameters and time, or season, of trapping produced results which appear to contradict the conclusions obtained with other forms of analyses and also results which appear to differ for the time periods examined. It is important in obtaining an overview of these results, to key on factors of biological significance. For example, while utilizing the total adult female population in analyses, as an indication of total reproductive potential of a population, proportion effects resulting from the nonpregnant component of the female population are introduced, thereby altering the biological significance of the results. That is, nonpregnant females may be of little biological significance in determining reproductive potential.

In this particular series of correlation analyses there is a lack of any consistent pattern of correlations that would indicate a significant correlation of pregnancy parameters with time. While significant correlations were obtained for a number of pregnancy parameters and time; it is believed, that these are due to such biases as a very large pre-implantation loss in a single population (Pinsonault, Jacob Road) and small sample sizes at some trapping sites.

Correlation of pregnancy parameters with distance from Windsor also produces results which differ from those obtained by other forms of statistical analysis. In this case, the differences may be, to some extent, explained by proportionate effects within the female population.

There is no significant correlation between the mean number of resorptions per pregnant female and distance from Windsor, however, significant negative correlations (-0.21, t = -0.69, -0.18, t = -0.63) are found between the number of pregnant animals per population and distance, and the total number of females per population and distance from Windsor. Therefore, while the number of animals decreases the farther from Windsor the trapping site was located the mean number of resorptions per pregnant female remained about the same thereby producing a relative increase in the mean number of resorptions per pregnant animal. To confirm that the significant correlations obtained were due to the proportion of pregnant and non-pregnant animals utilized for the analyses, mean number of resorbed embryos (per population) divided by the number of non-pregnant animals was compared to distance. As expected, significant positive correlation (0.34, t = 1.18) was obtained.

Also influencing these results, is the fact that some populations located furthest away from Windsor, such as Pinsonault, Jacob Road, and Caron were trapped during the winter and had small sample sizes and high prenatal loss or were shown to differ from other populations' pregnancy parameters by the use of SNK analysis.

Chi-square analysis revealed no significant differences in pregnancy parameters between populations from different geographic location. This confirms that identical factors are affecting all populations.

The two extremes of the sampling range differ greatly in some

aspects of the environment. The Harrow region lies directly in the path of industrial air pollutants brought from the North shore of the Detroit River by the prevailing winds of the region. Near Lake St. Clair, to the North-East, there is relatively little exposure to industrial air pollution. The lack of variation within Essex and Kent counties, of pregnancy parameters, especially resorptions, indicates, that at least some air pollutants, for example heavy metals, do not appreciably affect prenatal mortality in wild mice.

Estimates of Genetic Load

While the environment and parental genetic factors can influence litter size and survival, the genetic constitution of embryos, will also contribute to the amount of prenatal mortality detected in a population.

Embryonic lethals are known to act throughout the gestation period. The majority of these act after zygote implantation (Batten and Berry, 1967). Gluecksohn-Waelsch (1953) in a review on embryonic lethal genes in both invertebrates and vertebrates points out that identification of few lethal factors affecting early stages of development were reported because a) lethal factors affecting the very early stages of development are more difficult to detect and to study than those which act later in development, and b) early embryos exhibit the property of resisting or regulating damage during development. This latter property is similar to Waddington's concept of genetic homeostasis (Waddington, 1957).

There are, however, examples of lethals which act early in development. The Yellow lethal gene when present in the homozygous state in the mouse interferes with embryo implantation in the uterus (Robertson, 1942).

Several mutations in linkage group IX of the mouse influence early embryonic mortality and a number effect embryonic mortality much later in development than the Yellow gene (Bennett, 1964).

Embryos homozygous for allele $\underline{t}^{12}$ pass the morula stage of development (approximately day 4) but, degenerate before transforming to blastocysts. Those embryos homozygous for the recessive $\underline{t}^{0}$ allele do not produce normal extra embryonic ectoderm and embryonic ectoderm, and subsequently die by day 6 or 7 or gestation (Bennett, 1964).

In the case of brachyury (T), no abnormalities are found in homozygous T/T embryos up to the age of 8 days after fertilization. Between eight and ten days after fertilization irregularities of the neural folds and somites appear. These defects may not influence mortality though because Gluecksohn-Schoenheimer (1944) have shown that these same embryos do not develop umbilical connections with their mother. Embryos normally develop dependency on the placental circulation about day 10 after fertilization.

Genetic load is defined as the relative decrease in mean fitness of a population, with respect to the fitness it would have if all individuals in the population had the genotype with maximum fitness (Cavalli-Sforza and Bodmer, 1971). Genetic load is the price a

population must pay for maintaining deleterious recessive alleles in the heterozygous state within the gene pool of the population.

Since it has been shown that deleterious alleles may exist in populations, it would be expected, that post-implantation mortality should be high (particularly in inbreeding populations) if a large genetic load exists. In the Ontario samples mean post-implantation mortality was estimated to be 8.1 percent. This is similar to the range of post-implantation mortality values obtained by Batten and Berry (1967) of 2.5 and 17.1 percent and also their adjusted (dead embryos x 100/implants) range values of 1.2 to 10.7. Batten and Berry propose, that since these values are small, most post-implantation deaths in wild mice can be explained by newly arisen mutation, estimated to have a rate of at least 6 percent per zygote. Batten and Berry (1967) claim that most new mutations should be expressed in the heterozygous form. This hypothesis explains mortality due to dominants in heterozygotes, but also it could be possible to have mortality due to recessives maintained in the population but not accumulated due to population structure.

Maternal insufficiency, such as hormonal imbalance and embryo genotype may account for the remaining post-implantation mortality. Additional evidence in support of this hypothesis is presented in Batten and Berry's paper, but it is concluded, that genetic load is small in natural Mus musculus populations in Britain and in Ontario also.

Malformations in embryos should also indicate abnormal genetic constitution, but none was observed in this study, again indicating

that genetic load, in these populations, is small.

A higher number of individual detectable resorbed embryos occurred in litters greater than day 10 after fertilization than before day 10. More totally resorbed litters occurred in litters less than day 10 after fertilization than after day 10. This finding makes sense in terms of reproductive effort expended by the dam, however some correction is required since at least some totally resorbed litters are the result of maternal stress.

Correction is also required for the data, to account for a) embryonic death which occurred before day 10 in litters examined after day 10 and b) embryonic death which would have occurred later in gestation had the dams not been sacrificed, if the results are to be completely accurate.

Summary

Litter size, as determined by number of implants per female, varied among the populations the mean size being 5.97 ± 0.24. This value is similar to that obtained by Laurie (1946) and is within the range of 5.0 to 10.7 obtained in Batten and Berry's study (1967). Except for one population (Pinsonault, Jacob Road) there was little change in litter size throughout the year, as was found in the British populations. Litter size could be determined by selection acting on any one of the genetically controlled factors which influence litter size, for example, the number of eggs ovulated. Lack (1948) suggests, that

selection will favour those genotypes which give rise to the maximum number of new parents. Within any species however, the upper limit of actual litter size will be less than the theoretical maximum because embryonic and maternal mortality is increased in very large litters.

In this study, litter size was significantly positively correlated with maternal weight (used as a measure of size) this correlation is in agreement with Batten and Berry's (1967) findings.

Contrary to some reports in the literature, there were no statistically significant differences found between the right and left uterine horns for parameters such as live and resorbed embryo numbers. Mortality of embryos was spread randomly throughout the various positions within the uterus. This study suggests, that females with at least one resorbed embryo tended to have more than one resorbed and lighter or smaller females tended to have more resorbed embryos than heavier females.

Deleterious genetic factors, for example the embryonic lethal genes Yellow and Brachyury, cause considerable post-implantation mortality in wild and laboratory mice (Batten and Berry, 1967). If the arguments presented by Batten and Berry (1967) are utilized, then the relatively low levels of post-implantation mortality, seen in the populations utilized for this study, indicates that the "genetic load" of these populations is small.

There appears to be little variability of embryonic mortality within the study populations in terms of time of year the sampling was

done, and geographic location of the population. No significant differences were found among the populations for such pregnancy parameters as live, resorbed and implanted embryos. This lack of variation in turn suggests, that there is little, if any, influence by industrial air pollutants on prenatal mortality in wild-mouse populations in Southern Ontario.

There appeared, to be no pattern of association between pregnancy parameters and specific genotypes as determined by electrophoretic typing of a number of protein systems. It can only be concluded, that the loci examined in this study bear no influence on prenatal mortality in the mouse. Other protein loci not examined in the mouse may very well function in a regulatory role in determining prenatal mortality in the mouse.

Counts of corpora lutea on mice ovaries made by either macroscopic or serial section microscopic counting methods do not differ significantly.

When compared to data from Batten and Berry (1967) the present study shows lower pre-implantation death mean values but a greater range than that obtained for British samples. Overall prenatal mortality was only slightly lower in the Canadian populations than in the British samples.

In conclusion, this study has shown that populations of wild mice in Southern Ontario are remarkably similar to one another in terms of a number of pregnancy parameters and are quite similar to samples obtained from different environmental situations also.

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

Genetic variability as detected by electrophoresis or physical measurement has been shown to exist in substantial amounts in natural populations of Mus musculus. When response to the blood anticoagulant warfarin is examined in wild house mice from North America variability is also found. Whether an animal lives or dies after exposure to warfarin measures genetic variability in the animal. It is not known if the mode of inheritance of warfarin resistance is the same in North America and British populations, but a resistance system does exist. At most, two different loci appear to cause the same resistance phenotype in more than two populations of the same species. The simplest hypothesis to explain the similarity in proportion of resistant animals in North America and Britain is that a homologous, inherited resistance system is present. Obviously, more work is required to elucidate the mode of inheritance of warfarin resistance in different wild house mice and to determine the mechanism by which resistance is brought about.

Using the Quick Prothrombin Time Test, no significant differences in blood clotting time could be detected between groups of inbred or wild house mice or between warfarin susceptible or resistant animals. While inter-individual variation in clotting time did exist, the insensitivity of the test procedure may have prevented these differences from being interpreted as significant.

When reproductive patterns in natural populations of Mus musculus were examined to determine pre- and post-implantation mortality estimates, minor variations were detected among the populations for some pregnancy parameters such as number of implants and number of corpora lutea per female. On the whole however, populations of wild mice in Southern Ontario were remarkably similar to one another in terms of the pregnancy parameters examined. Estimates of pre- and post-implantation mortality in Ontario were similar to those presented for British samples.

In conclusion, variability in response to warfarin was shown to exist in North American house mouse populations, but little variation existed, in blood clotting times in inbred and wild mice, and in pre- and post-implantation mortality in wild mice. A very interesting point is the similarity of Ontario and British populations for the parameters examined in these studies.

APPENDIX A

DETAILED DATA ON TOXICITY TESTING, ANIMAL WEIGHTS, ELECTROPHORETIC TYPING OF GENOTYPES, AND AUTOPSY FINDINGS.

Table 34: Toxicity testing of Mus musculus from South Western Ontario, New York State, and Britain using 0.025 percent warfarin in the diet

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
Belanger	77-124	F	13.7	7.2 (0.8)	131.4	9	do	ab
Dover Township	77-121	M	23.1	41.3 (2.8)	447.0	15	S1	a
	77-119	M	17.8	60.2 (2.9)	845.5/21D	24	do	a
	77-118	M	14.0	14.0 (1.3)	218.0	11	F	a
Big Pt. XII Dover Township	77-93	F	18.5	11.3 (1.9)	152.7	6	S1	b
Bondy	77-1284	M	17.4	6.4 (0.6)	92.0	10	S1	b
Harrow	77-1292	M	17.2	11.9 (1.0)	172.9	14	S1	ab
	77-1290	M	18.2	19.0 (1.4)	261.0	14	S1	ab
Caron	77-187	M	16.3	13.0 (1.3)	199.4	10	S1	a
Stoney Point	77-217	M	14.1	14.8 (1.9)	262.4	8	do	a
	77-199	F	15.8	59.1 (2.8)	935.1	Survived	S1	a
	77-213	M	19.7	58.2 (2.8)	738.6/21D	25	S1	a
Comartin	77-62	F	19.0	27.5 (2.5)	361.8	11	S1	a
Stoney Point	77-60	F	17.5	65.1 (3.1)	930.0	Survived	S1	a

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Gpi-1
15th Concession Dover Township	77-575	M	16.8	31.4 (2.0)	467.3	16	S1	ab
	77-522	M	18.0	59.7 (2.8)	829.0	Survived	S1	a
	77-519	M	16.0	59.8 (3.8)	934.4	Survived	do	b
	77-516	M	16.6	18.5 (1.7)	278.6	11	S1	a
	77-578	M	21.2	64.9 (3.1)	765.3/21D	24	S1	a
	77-587	F	15.6	7.2 (0.9)	115.4	8	S1	a
	77-584	M	16.7	18.2 (1.4)	272.4	13	do	a
	77-581	M	17.9	25.0 (1.9)	349.2	13	do	a
	77-582	F	14.3	21.2 (1.6)	370.6	13	S1	a
	77-580	F	17.9	5.5 (0.7)	76.8	8	S1	a
	77-527	M	15.8	6.8 (0.8)	107.6	8	S1	a
	77-532	M	15.2	10.4 (1.0)	171.0	10	S1	b
	77-585	M	19.6	22.9 (1.1)	292.1	21	S1	ab
	77-583	F	16.1	59.4 (2.8)	922.4	Survived	S1	ab
	77-526	M	16.4	8.0 (0.7)	122.0	11	S1	ab
	77-947	F	15.5	14.0 (1.8)	225.8	8	S1	a
7th Concession Dover Township	**77-496	M	12.9	123.3 (2.9)	2389.5	Survived/42D	F	ab
	**77-500	M	13.2	129.1 (3.1)	2445.1	Survived/42D	do	ab
	77-483	M	17.9	33.1 (3.3)	462.3	10	do	a
	77-471	M	14.0	12.9 (1.3)	230.4	7	do	ab
	77-501	M	20.1	63.1 (3.0)	784.8	Survived	do	a
	77-447	M	16.4	70.2 (3.3)	1070.1	Survived	S1	a
	77-455	M	18.7	67.2 (3.2)	898.4	Survived	S1	ab
	77-464	M	18.7	81.1 (3.9)	1084.2	Survived	S1	a
	77-484	M	16.6	21.8 (2.0)	328.3	11	do	ab

** Excluded from calculations

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
7th Concession	77-462	M	15.0	25.1 (1.8)	418.3	14	do	a
Dover Township	77-458	M	17.1	26.5 (1.9)	387.4	14	do	ab
	77-503	M	12.0	14.8 (1.0)	308.3	14	do	a
Faubert	77-2251	F	17.7	35.3 (2.2)	498.6	16	do	a
Dover Township								
Grand Pt. IX	77-4	F	17.4	41.4 (2.8)	594.8	15	S1	a
Dover Township	77-2	M	20.9	79.3 (3.8)	948.6	Survived	do	a
Houle	77-867	M	17.7	49.1 (2.7)	693.5	18	S1	a
Stoney Point								
Hutchinson	77-2249	M	19.6	75.7 (3.6)	965.6	Survived	F	a
Harrow								

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
Maitre	77-279	M	18.2	11.7 (1.1)	160.7	11	S1	a
	77-273	M	18.7	22.9 (2.1)	306.1	11	S1	a
	77-267	M	17.4	37.7 (2.0)	541.6	19	do	a
	77-271	M	21.1	69.8 (3.3)	827.0	Survived	do	a
	77-272	M	22.7	69.6 (3.3)	766.5	Survived	do	a
	77-361	M	17.2	48.7 (2.6)	707.8	19	S1	a
	77-368	F	12.9	55.1 (2.6)	1067.8	Survived	do	a
	77-322	M	18.7	62.4 (3.0)	834.2/21D	23	S1	a
	77-319	M	13.3	16.8 (1.3)	315.8	13	do	a
	77-340	M	17.7	23.6 (2.6)	333.3	9	S1	a
	77-354	M	14.3	15.0 (1.7)	262.2	9	F	a
	77-366	M	22.6	80.6 (3.8)	891.6	Survived	do	a
	77-338	M	13.4	53.4 (2.5)	996.3	Survived	F	a
	77-362	M	15.5	61.2 (2.9)	987.1/21D	26	do	a
	77-376	M	20.6	57.8 (2.8)	701.4/21D	22	do	a
	77-325	M	20.0	65.9 (3.1)	823.8/21D	23	do	a
Martin, D. Harrow	77-2203	F	15.2	20.2 (1.7)	332.2	12	do	a
	77-2202	F	16.6	69.3 (3.3)	1043.7	Survived	do	a
Martin, K.P. Harrow	77-2121	M	14.9	69.4 (3.3)	1164.4	Survived	S1	a
	77-2126	M	16.4	10.4 (0.9)	158.5	12	S1	a
McCallum	77-2235	M	15.4	24.2 (1.9)	392.8	13	do	a
	77-2240	M	13.9	14.8 (1.8)	266.2	8	F	ab

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
Pigeon	77-1094	F	17.6	2.0 (0.3)	28.4	7	S1	b
Harrow	77-1219	M	19.1	29.3 (2.2)	383.5	13	do	ab
	77-1085	M	16.1	13.3 (2.7)	206.5	5	do	--
Price	77-386	M	29.6	29.6 (3.1)	338.7	13	S1	b
Central Essex County	77-391	M	19.3	44.8 (2.6)	580.3	17	S1	ab
	77-413	M	18.0	16.6 (2.1)	222.2	8	S1	ab
	77-382	M	15.3	32.8 (3.3)	535.9	10	S1	a
	77-404	M	16.6	49.5 (2.1)	736.6/21D	24	S1	a
	77-398	M	20.5	89.9 (4.3)	1096.3	Survived	S1	a
	77-400	F	18.1	58.4 (2.8)	806.6	Survived	do	a
	77-385	M	15.2	30.9 (2.8)	508.2	11	S1	ab
	77-415	F	13.8	15.7 (1.4)	284.4	11	do	b
	77-384	M	24.5	74.1 (3.5)	756.1/21D	23	do	a
	77-402	M	29.2	9.1 (2.3)	77.9	4	S1	a
Richardson	77-1275	M	17.5	24.8 (2.1)	354.3	12	do	b
	77-1280	M	24.2	20.6 (1.7)	212.8	12	S1	a
	77-1270	M	15.2	76.5 (3.6)	1250.0	Survived	S1	b
	77-1261	M	19.6	44.4 (2.8)	566.3	16	S1	a
	77-1252	M	21.3	75.5 (3.6)	886.2	Survived	S1	ab
	77-1251	M	22.0	99.6 (4.7)	1131.8	Survived	S1	ab
	77-1277	M	23.5	24.5 (1.9)	260.6	13	S1	b

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
Rocheleau	77-107	M	16.5	45.9 (2.7)	695.4	17	S1	ab
	77-109	M	22.1	16.3 (2.7)	184.4	6	S1	a
Central Essex County	77-69	M	18.6	22.6 (2.8)	303.8	8	S1	a
	77-37	M	15.0	22.1 (2.0)	368.3	11	F	a
Controls								
Bondy	77-996	M	16.7	49.8 (2.4	---	Survived	S1	a
Pigeon	77-1072	M	24.6	55.2 (2.6)	---	Survived	S	b
Ouellette	77-439	M	15.8	58.2 (2.8)	---	Survived	do	a

Continued...

Table 34: Continued

Population	Mouse Number	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Electrophoresis Gpi-1
New York State								
Baldwin	511, 594		22.8	15.1 (2.2)	165.6	7	F	a
Decker	258, 300	F	23.0	16.6 (1.5)	180.4	11	S1	ab
Domin	540, 170	F	18.8	27.9 (1.6)	371.0	17	do	b
	537, 5050	F	16.4	40.7 (2.0)	620.4	20	do	a
	544	M	31.1	26.9 (2.7)	216.2	10	do	a
Michael	538, 4030	F	20.6	10.3 (1.5)	125.0	7	do	a
Pierson	P.C. 4000	F	27.6	41.6 (2.0)	376.8	Survived	S1	a
	491, 5000	F	18.6	29.1 (1.5)	391.1	19	do	a
Roemer	520, 1500	F	18.5	17.4 (1.7)	235.1	10	F	a
Ross	533, 7000	F	19.4	11.3 (1.4)	145.6	8	F	a
Shield	492	M	25.6	65.7 (3.1)	641.6	Survived	do	a
	366	F	21.0	23.9 (1.6)	284.5	15	F	ab
	400	F	21.5	14.5 (0.8)	168.6	18	F	a
Control								
Domin	565, 1920	F	22.4	47.6 (2.3)	---	Survived	do	a
Shield	377	F	22.8	56.3 (2.7)	---	Survived	F	a

Continued...

Table 34: Continued

Mouse Number	Generation	Sex	Weight	Amount Bait Eaten (gm) Total (Per Day)	Warfarin Dose (mg/kg)	Day of Death	Electrophoresis Hbb	Gpi-1
[PBI ($c^e c^e$), MAFF ($c^e c^e$)]								
155	BC_1	M	21.8	50.7 (2.4)	581.4	Survived	S1	b
179	BC_1	F	24.8	84.8 (4.0)	854.8	Survived	S1	b
184	BC_1	F	26.8	78.9 (3.8)	736.0	Survived	S1	b
112	BC_1	F	16.9	55.5 (2.6)	821.0	Survived	S1	b
142	F_1	M	23.0	22.5 (2.0)	244.6	11	S1	b
143	F_1	M	22.4	31.3 (1.8)	349.3	17	S1	b
144	F_1	M	25.0	13.3 (2.2)	133.0	6	S1	b
145	F_1	M	19.7	16.1 (1.2)	204.3	13	S1	b
146	F_1	F	18.7	18.7 (2.8)	796.8	Survived	S1	b
20	F_1	F	23.6	46.1 (2.2)	488.3	Survived	S1	b
21	F_1	F	23.1	30.4 (2.0)	329.0	15	S1	b
147	F_1	F	20.6	59.6 (2.8)	723.3	Survived	S1	b
123	F_1	M	23.0	33.5 (1.8)	364.1	19	S1	b
107	F_1	F	15.6	43.4 (2.0)	695.5/21D	22	S1	b
105	F_1	F	19.7	40.3 (1.9)	511.4	Survived	S1	b
106	F_1	F	19.8	58.9 (2.8)	743.7	Survived	S1	b
120	F_1	F	24.1	51.8 (2.5)	536.7	Survived	S1	b
121	F_1	F	22.5	48.9 (2.3)	543.3	Survived	S1	b
130	F_1	F	22.0	42.3 (2.0)	480.7	Survived	S1	b
131	F_1	F	23.7	44.5 (2.1)	469.4	Survived	S1	b
132	F_1	F	22.9	38.5 (1.8)	420.3	Survived	S1	b
133	F_1	F	20.7	18.4 (1.4)	222.2	13	S1	b
80	F_1	F	25.4	63.1 (3.0)	621.1	Survived	S1	b
82	F_1	F	29.4	75.1 (3.6)	638.6	Survived	S1	b
164	BC_1	F	16.2	48.5 (2.3)	748.4	Survived	S1	b
167	BC_1	F	19.0	55.9 (2.7)	735.5	Survived	S1	b
125	BC_1	M	22.3	62.7 (3.0)	702.9	Survived	S1	b

Table 35: Number of animals of specific weights and weight range classes, for animals utilized in toxicity testing

Weight (gm)		0-11	12	13	14	15	16	17	18	19	20	21	22	23	24	25-28	29
Ontario Wild Sample																	
Animals Which Survived Toxicity Testing	M	0	1	2	1	1	2	0	3	1	3	2	3	0	0	0	2
	F	0	1	0	0	1	2	1	1	0	0	0	0	0	0	0	0
		0 - 15 gm					16 - 20 gm					21 - 30 gm					
	M	5* (23.81)**					9 (42.86)					7 (33.33)					
	F	2 (33.33)					4 (66.67)					0					
	M & F	7 (25.92)					13 (48.15)					7 (25.93)					
Animals Which Died During Toxicity Testing	M	0	1	2	3	8	11	11	5	6	2	1	1	2	2	0	0
	F	0	0	2	1	3	0	4	1	1	0	0	0	0	0	0	0
		0 - 15 gm					16 - 20 gm					21 - 30 gm					
	M	14 (25.45)					35 (63.64)					6 (10.91)					
	F	6 (50.00)					6 (50.00)					0					
	M & F	20 (29.85)					41 (61.19)					6 (8.96)					

Sample Size (N) = 94
* Number of Animals
** Percent

Table 35: Continued.

Weight (gm)		0-15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
New York Sample																		
Animals Which Survived Toxicity Testing	M	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
			(0 - 20)					(21 - 25)					(26 - 31)					
	M			0*				1 (50.0)**					1 (50.0)					
	F			0				0					0					
	M & F			0				1 (50.0)					1 (50.0)					
Animals Which Died During Toxicity Testing	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	F	0	1	0	3	1	1	2	1	1	0	0	0	0	0	0	0	0
			(0 - 20)					(21 - 25)					(26 - 31)					
	M			0				0					1					
	F			6 (60.0)				4 (60.0)					0					
	M & F			6 (54.54)				4 (36.36)					1 (9.09					

Sample Size (N) = 13
* Number of Animals
** Percent

Table 35: Continued

Weight (gm)		0-14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
PBI($c^e c^e$)/MAFF($c^e c^e$) F_1 & BC_1																		
Animals Which Survived Toxicity Testing	M	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	F	0	0	2	0	1	3	1	0	3	2	2	1	1	0	0	1	0
				(0 - 20)						(21 - 25)					(26 - 30)			
	M			0*						2 (100.0)					0			
	F			7 (41.18)**						8 (47.06)					2 (11.76)			
	M & F			7 (36.84)						10 (52.63)					2 (10.53)			
Animals Which Died During Toxicity Testing	M	0	0	0	0	0	0	1	0	1	2	0	1	0	0	0	0	0
	F	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
				(0 - 20)						(21 - 25)					(26 - 30)			
	M			1 (20.00)						4 (80.00)					0			
	F			2 (66.67)						1 (33.33)					0			
	M & F			3 (37.50)						5 (62.50)					0			

Sample Size (N) = 27
* Number of Animals
** Percent

Table 36: Summary of electrophoretic data

		Electrophoresis								
		Hbb				Gpi-1				Alb-1
	N	$\frac{Hbb^s}{Hbb^s}$	$\frac{Hbb^s}{Hbb^F}$	$\frac{Hbb^F}{Hbb^F}$	Total	$\frac{Gpi\text{-}1^a}{Gpi\text{-}1^a}$	$\frac{Gpi\text{-}1^a}{Gpi\text{-}1^b}$	$\frac{Gpi\text{-}1^b}{Gpi\text{-}1^b}$	Total	
Ontario Wild Mouse Sample	94	53	34	7	94	62	21	10	93	All fast
Genotypic Frequency		0.56	0.37	0.7		0.67	0.22	0.11		
Warfarin Resistant Subgroup	25	12	10	3		17	6	2		All fast
*Expected		14.1	9.0	1.9		16.7	5.6	2.7		
X^2		0.31	0.11	0.64		0.00	0.03	0.18		
Genotypic Frequency		0.48	0.40	0.12		0.68	0.24	0.08		

* Expected values based on no correlation between genotype and warfarin resistance

Table 37: Autopsy findings

Population	Mouse Number	Before Death	External at Death	Hemorrhage Abdomen	G.I. Tract	Thorax
Belanger	F124	-	+ (Digits)	+ (1)	-	-
	M121	+ (Mouth)	-	+ (3)	-	-
	M119	+ (Nose)	+ (Nose)	+ (1)	+ (1)	-
	M118	-	-	-	+ (3)	-
Big Pt. XII	F93	+ (Nose, ear)	+ (Nose, ear)	-	-	-
Bondy	M1284	-	-	+ (3)	-	-
	M1292	-	-	+ (1)	+ (2)	-
	M1290	-	-	+ (3)	-	+ (2)
Caron	M187	-	-	+ (1)	-	-
	M217	-	-	+ (1)	-	+ (1)
	M213	-	-	+ (1)	+ (1)	-
Comartin	F62	-	-	+ (3)	-	-

Continued...

Table 37: Continued

Population	Mouse Number	Before Death	External at Death	Hemorrhage Abdomen	G.I. Tract	Thorax
15th Concession	M575	-	-	+ (1)	+ (1)	-
	M516	+ (Nose)	+ (Digits)	-	+ (3)	-
	M578	-	-	+ (1)	-	-
	F587	-	+ (Nose)	+ (3)	-	-
	M584	-	-	+ (3)	-	-
	M581	-	-	+ (3)	-	+ (1)
	F582	-	+ (Mouth)	+ (3)	+ (3)	-
	F580	-	-	-	-	- *
	M527	-	+ (Ear, nose)	+ (3)	-	-
	M532	+ (Ear)	+ (Ear)	+ (3)	-	+ (1)
	M585	+ (Nose)	+ (Nose)	+ (3)	-	-
	M526	-	-	+ (1)	+ (1)	-
	F947	-	-	+ (1)	-	-
7th Concession	M483	-	-	+ (1)	+ (3)	-
	M471	-	-	+ (1)	+ (1)	+ (1)
	M484	+ (Nose)	+ (Nose)	+ (1)	+ (3)	+ (1)
	M462	-	-	+ (1)	+ (1)	-
	M458	-	-	+ (1)	+ (1)	-
	M503	-	-	+ (1)	+ (1)	-
Faubert	F2251	-	-	+ (1)	+ (1)	-
Grand Pt. IX	F4	-	+ (Nose, mouth)	+ (3)	-	+ (3)
Houle	M867	+ (Nose)	-	+ (2)	+ (2)	-

Continued...

Table 37: Continued

Population	Mouse Number	Before Death	External at Death	Hemorrhage Abdomen	G.I. Tract	Thorax
Maitre	M279	-	-	+ (3)	-	-
	M273	-	-	+ (1)	-	+ (1)
	M267	-	-	+ (3)	-	-
	M361	-	-	+ (3)	-	-
	M322	-	-	-	-	- *
	M319	-	-	+ (1)	+ (1)	+ (1)
	M340	-	+ (Digits)	+ (2)	-	-
	M354	-	-	+ (2)	-	-
	M362	+ (Ear)	-	+ (3)	+ (3)	-
	M376	-	-	+ (1)	+ (1)	-
	M325	-	-	+ (1)	+ (1)	+ (1)
Martin, D.	F2203	-	-	+ (1)	+ (1)	-
Martin, K. P.	M2126	-	-	+ (3)	-	+ (3)
McCallum	M2235	-	-	+ (1)	+ (1)	-
	M2240	-	-	+ (1)	+ (1)	-
Pigeon	F1094	-	+ (Digits)	+ (0)	-	- **
	M1219	-	-	+ (0)	-	- **
	M1085	-	-	+ (3)	-	-

* No sign of bleeding
** Very slight bleeding

Continued...

Table 37: Continued

Population	Mouse Number	Before Death	External at Death	Hemorrhage Abdomen	G.I. Tract	Thorax
Price	M386	-	-	+ (1)	+ (1)	-
	M391	+ (Nose)	+ (Face, digits)	+ (3)	+ (2)	-
	M413	+ (Mouth)	-	+ (1)	-	-
	M382	-	+ (Digits)	+ (2)	-	+ (1)
	M404	-	-	+ (3)	-	-
	M385	+ (Nose)	+ (Nose)	-	-	+ (3)
	F415	-	-	-	+ (1)	-
	M384	-	-	+ (1)	-	-
	M402	-	-	+ (1)	+ (1)	-
Richardson	M1275	-	-	-	-	- *
	M1280	-	-	+ (1)	-	-
	M1261	+ (Nose)	-	+ (1)	-	-
	M1277	-	-	+ (3)	-	-
Rocheleau	M107	-	-	+ (1)	-	+ (1)
	M109	-	+ (Digits)	+ (1)	-	-
	M69	-	-	+ (1)	-	-
	M37	-	-	+ (1)	-	-

Continued...

Table 37: Continued

Population	Mouse Number	Before Death	External at Death	Hemorrhage Abdomen	G.I. Tract	Thorax
Baldwin	F511, 594	-	-	+ (1)	+ (1)	-
	F258, 300	-	-	+ (1)	-	-
	F540, 170	-	-	-	+ (2)	+ (2)
	F537, 5050	-	-	+ (3)	-	-
	M544	-	-	+ (1)	+ (3)	-
Michael	F538, 4030	+ (Digits,ear)	-	-	+ (3)	-
Pierson	F491, 5000	-	-	+ (1)	+ (3)	-
Roemer	F520, 1500	-	-	+ (1)	+ (1)	+ (1)
Ross	F533, 7000	-	-	+ (3)	-	-
Shield	F366	-	+ (Digits)	-	+ (1)	-
	F400	-	-	-	+ (1)	-
PBI/MAFF F_1 & BC_1	M142	-	-	+ (1)	-	-
	M143	-	+ (Digits)	+ (1)	-	-
	M144	-	-	-	-	- *
	M145	-	-	-	-	- *
	F21	-	-	+ (1)	+ (1)	-
	M123	-	-	+ (1)	-	-
	F107	-	-	+ (1)	-	-
	F133	-	+ (Digits)	+ (1)	-	-

APPENDIX B

DETAILED DATA ON PRE- AND POST-IMPLANTATION MORTALITY IN FEMALES WITH LIVE AND 100 PERCENT RESORBED LITTERS.

Table 38: Estimates of pre-implantation and total prenatal loss for females, including those with 100% resorbed litters

Population	Implants Per Female with Live & 100% Rd.	Corpora Lutea Per Female with Live & 100% Rd. Implants	Pre-Impl. Loss Per Female	Prenatal Loss Per Female (%)
Caron	7.25 ± 1.83 (8)*	---	---	---
Price, N.&.S	6.67 ± 2.12 (9)	---	---	---
15th Concession	6.33 ± 1.87 (12)	6.42 ± 1.73 (12)	0.08 (1.3%)	11.7%
7th Concession	6.92 ± 1.79 (24)	7.38 ± 2.82 (24)	0.46 (6.2%)	15.2%
Ouellette	6.67 ± 3.52 (12)	8.75 ± 3.36 (12)	2.08 (23.8%)	34.5%
Houle	6.12 ± 1.19 (24)	6.50 ± 1.10 (24)	0.38 (5.8%)	15.4%
Bondy Lt.	5.00 ± 1.67 (6)	6.17 ± 2.40 (6)	1.67 (18.9%)	18.9%
Pigeon	5.54 ± 1.37 (87)	6.13 ± 1.58 (89)	0.59 (9.6%)	14.2%
Bondy, SR & NR.	7.83 ± 1.33 (6)	9.33 ± 2.34 (6)	1.50 (16.1%)	23.2%
McKim	5.53 ± 1.28 (17)	6.35 ± 1.41 (17)	0.82 (12.9%)	23.1%

Continued...

Table 38: Continued

Population	Implants Per Female with Live & 100% Rd.	Corpora Lutea Per Female with Live & 100% Rd. Implants	Pre-Impl. Loss Per Female	Prenatal Loss Per Female (%)
Pinsonault #6	6.67 ± 1.53 (3)	9.33 ± 2.49 (3)	3.33 (35.7%)	35.7%
Pinsonault J.R.	8.00 ± 1.73 (5)	12.80 ± 3.87 (5)	4.80 (37.7%)	39.4%
Pigeon - Apr.	5.90 ± 1.29 (10)	8.70 ± 1.90 (10)	2.80 (32.2%)	32.3%

* Number of Females

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