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Title: GENETIC ASPECTS OF LEAD TOXICITY IN LABORATORY POPULATIONS OF GUPPIES (POECILIA RETICULATA)

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The heritability of resistance to death from the effects of lead in laboratory guppy populations was determined from comparisons between progeny produced in a nested breeding experiment and from mass selection experiments. Resistance was measured by the time to death. Heritabilities of resistance to lead were estimated to be 0.26 and 0.57 from comparisons of progeny resistance. Realized heritability estimates ranged from 0.28 to 0.68.

Uptake studies following exposure of guppies to lead contaminated solutions showed that greater amounts of lead were concentrated in the head region than in other body tissues. Uptake rates were greater in lead resistant groups than in control groups.

Genetic Aspects of Lead Toxicity in Laboratory Populations of Guppies (*Poecilia reticulata*)

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GENETIC ASPECTS OF LEAD TOXICITY IN LABORATORY POPULATIONS OF GUPPIES (*POECILIA RETICULATA*)

INTRODUCTION

It has been demonstrated in a variety of plant and animal populations that their abilities to tolerate the presence of environmental contaminants often increases with time (Abedi, 1963; Bradshaw *et al.*, 1965; Crow, 1957; Ferguson, 1967). Selection for more tolerant phenotypes apparently results in an alteration of genetic structure leading to an increase in the mean resistance of the members of these populations.

Heavy metal toxicity in biological systems and concentration in tissues are well documented (Carpenter, 1927; Hannerz, 1968; Whitton, 1970) but the genetic effects of these substances have only recently been demonstrated. Tolerance to lead and copper has been shown to be an inherited characteristic for populations of grasses in mining areas (Bradshaw *et al.*, 1965; Urquhart, 1970). Results obtained in factorial mating experiments (Blanc, 1973; McIntyre and Blanc, 1973) indicate that resistance to the lethal effects of methyl mercury is approximately 50 percent heritable for steelhead (*Salmo gairdneri*).

An important physiological consideration is the manner in which increased resistance is attained. Factors preventing the

concentration of contaminants in the body, as well as those which facilitate the storage of these materials in various tissues, may result in increased resistance. If resistant individuals can tolerate toxicants that are concentrated in the body tissues, a threat to predator populations could exist.

Irrespective of the magnitude of contamination, affected environments are rarely devoid of life. Consequently, genetic studies are necessary in order to estimate any short-term evolutionary changes that may result from environmental contamination. Accordingly, the objectives of this study were:

1. To determine the heritability of resistance to death from the effects of lead in an experimental population of fish (the guppy, Poecilia reticulata).

2. To determine whether mating experiments yield heritability estimates that are comparable to those obtained by mass selection.

3. To investigate the manner in which increased resistance, if heritable, is attained.

Specifically, experiments were designed to answer the question: is there a correlation between increased resistance and the amount of lead that is concentrated in the body tissues?

MATERIALS AND METHODS

Experimental Animals

Common guppies (Poecilia reticulata) were chosen as the test fish. Guppies are viviparous and, under the rearing conditions used in this study, had a gestation period of approximately 22 days. Sexual maturity occurred at an average age of 2.5 months. An initial brood stock of more than 200 individuals was used. These fish were maintained in a 50 gallon aquarium at 24°C that contained aerated, dechlorinated water. Green plants Ceratophyllum sp. which floated near the surface provided hiding places for the offspring. Tubifex worms, frozen brine shrimp, Oregon Moist Pellet and Tetramin Flakes constituted a varied diet. This system yielded a continuous supply of young fish (fry) which could be removed at predetermined intervals to provide experimental groups of individuals of approximately the same age.

Treatment Solutions

Two methods for the treatment of experimental fish in solutions that contained lead were used. The first treatment method was a standing water bioassay (S. W. B.) with lead acetate in dechlorinated water. The second treatment method was a flowing water bioassay

(F.W.B.). A concentrated solution of lead acetate from a Mariot Bottle provided the desired final concentrations in these constantly renewed solutions.

From the results of preliminary standing water bioassays with lead acetate, the concentration which killed 50 percent of an experimental group in about 48 hours was selected for use in the experiments. Characteristics of the dechlorinated water supply which was used in these experiments are described in Appendix I.

Because of the low solubility of lead, standard lead acetate solutions were made with distilled water at a low pH. It was then necessary to add increments of 4 N KOH to produce a final pH of 6.1 - 6.2 without the formation of a precipitate. By increasing the ionic strength in this manner, an ionic environment was created which held lead in solution.

In the standing water treatments, the pH of the dechlorinated water supply was adjusted to 6.2. Higher pH's resulted in the formation of a precipitate. No pH adjustments were made to the water supply used in the flowing water treatment. In all treatments, water temperature was approximately 20 - 22^{o}C.

Heritability Estimates

The phenotype (P) of an individual is composed of a genetic

component (G) and an environmental component (E) such that,

$$P = G + E$$ (for instance see Falconer, 1960; Pirchner, 1969)

It follows that the phenotypic variance (V_P) has genetic (V_G) and environmental (V_E) components. The genetic variance may be further divided into additive (V_A) and non-additive (V_D) components. Heritability (h^2) is defined as the ratio of additive genetic variance to the total phenotypic variance:

$$h^2 = \frac{V_A}{V_P}$$

The additive genetic variance is the chief cause of resemblance between relatives and can be estimated from breeding experiments (Becker, 1967). One such experiment, Breeding Design I of Comstock and Robinson (1952), was used here. In this nested design, each male is mated to n females and no female takes part in more than one mating. Therefore, m males and mn females produce mn progeny families. In the experiment described herein, each of 20 males was mated to five females to produce a maximum of 100 families.

A trough comprising 20 permanent compartments was constructed of plexiglass such that each compartment had a drain and an inflow of water.

Offspring groups from the brood tank were transferred to separate aquaria. As soon as males could be identified (development of elongated anal fin) they were removed from the aquaria. This procedure permitted isolation of a large group of virgin females. These females were reared to the age of nine months at which time they were put into compartments of the trough system (described above) in random lots of five per compartment. Twenty males were randomly chosen from a variety of sources and a single male was placed with each of the 20 groups of five females for a ten day "fertilization" period. Subsequently, dividers were installed in each of the 20 compartments to separate each female. Thereby, a total of 100 cells each containing one female were produced. Each cell contained approximately one gallon of water and received about 10 ml dechlorinated water per minute at 24°C. Ceratophyllum sp. was placed into each cell to protect the young from their predatory mothers.

Potentially, 100 full sibling families (five per male) could be generated by these matings. Since all of the females did not produce offspring at the same time however, two experiments (A and B) were performed:

A. Thirty-two full sibling families (two families for each of 16 males) were exposed to the treatment solution in groups of ten fry per female (20 per male).

B. Sixteen full sibling families (two families for each of

eight males) were exposed to the treatment solution in groups of 20 fry per female (40 per male).

The full sibling families used in these experiments were removed from the plexiglass system described above by siphoning the groups into separate collecting containers. For both experiments A and B, collection of the families was accomplished over two, separate, ten day time periods to minimize any weight differences between families. All families were assigned to cylindrical cells in an enameled trough according to a random treatment scheme (one family per cell). The cells were made from 2 in. P. V. C. pipe cut to a length of 2 in. These cells were cemented together and closed at the bottom by a continuous plastic screen. Glass rods were placed beneath the screen to elevate the cells above the bottom of the trough. This facilitated an equal exposure of the fry to the treatment solution. The families in both experiments were treated in a standing water bioassay at 7 mg/l Pb in 30 liters of dechlorinated water. The time to death for the individuals comprising the full-sibling families was recorded. Control fish were maintained for both experiments under similar, uncontaminated conditions.

Procedures for the analysis of variance and interpretation of variance components (Becker, 1967) are presented in Appendix II. The additive genetic variance is estimated from the covariance between male, half-sibling families. Assumptions included in

these analyses (Becker, 1967; Falconer, 1960) are:

1. No autosomal linkage of genes affecting the observed trait.

2. No interference with segregation and independent assortment of the chromosomes.

3. The inbreeding coefficient of the base population is zero.

4. Natural selection does not alter the estimated heritability between the time that the fish were treated and the time that they reach maturity.

To evaluate the validity of the heritability estimates obtained from the above analyses, several estimates of the "realized" heritability for resistance to lead were determined using methods described by Falconer (1960).

The response (R) to selection depends upon the heritability of an attribute (h^2), the degree of variation in an attribute (σ_p) and the intensity of selection (i):

$$R = i \sigma_p h^2,$$

where:

R = Mean of offspring from selected parents minus mean of all adults before selection.

$$i = \frac{\text{Mean of group selected minus Mean of all adults before selection}}{\sigma_p}$$

σ_p = Phenotypic standard deviation of the attribute.

h^2 = Heritability estimate for a particular attribute.

Thus,

$$h^2 = \frac{R}{(i)\,\sigma_p} \quad .$$

The response measured in this study was the difference between mean time to death of offspring from selected parents and mean time to death of all individuals before selection (Figure 1). Accordingly, heritability was estimated by measuring the response to selection in a test population as compared to a control population (where all members could be killed) in which no selection has occurred.

Two such selection experiments (1 and 2) were performed to estimate the realized heritability of lead resistance in guppies. For both experiments, large numbers of juvenile fish of approximately the same age were accumulated. Both "starting" populations will hereafter be referred to as foundation generations 1 and 2.

The first foundation generation was comprised of 2000 guppy fry approximately ten days of age. Of these, 1700 were treated at 5 mg/l Pb in 62 liters dechlorinated water (S. W. B.). The remaining 300 fry were maintained as a control population.

The second foundation generation consisted of 1200 fish which were reared to the age of two months prior to treatment. Of these, 1000 experimental fish were treated at 15 mg/l Pb in 45 liters

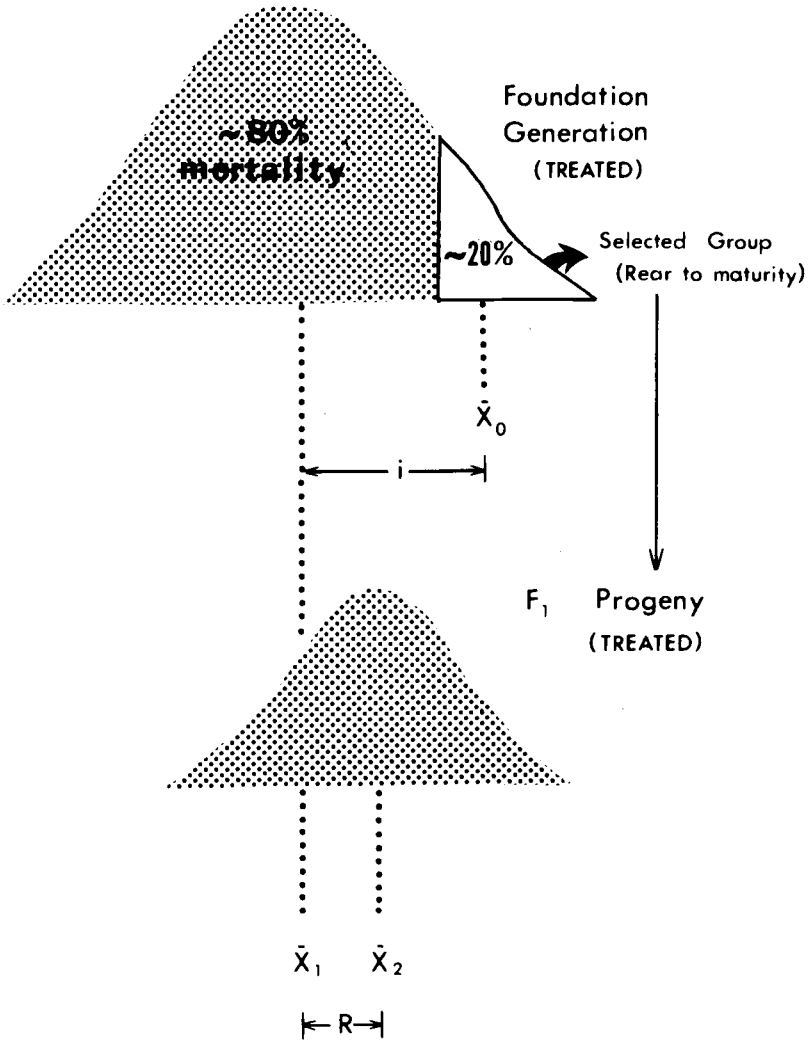

Figure 1. Method used to obtain realized heritability estimates of resistance to lead. An approximation of $\bar{X}_1$ was obtained following treatment of a F_1 control group. $\bar{X}_2 - \bar{X}_1$ gives the response to selection.

(S. W. B.). The remaining 200 fish served as a control population. Glass aquaria were used as the treatment containers in both experiments. Since the efficiency of heritability estimates is shown to be optimum when from 10 to 20 percent of a foundation population is selected (Soller and Genizi, 1967), the experimental fish from the two foundation generations were held in lead contaminated solutions until approximately 80 percent were dead. The survivors (18.05 and 21.9 percent of foundation generations 1 and 2, respectively) were retained as progenitors of the F_1 generations. The F_1 progeny were then exposed to lead contaminated solutions at the same age as were their parents and the time to death of each offspring was recorded. The mean time to death of progeny from the unexposed, control parents provided a basis from which the difference in mean time to death between "resistant" and "susceptible" fish, or the response to selection, could be determined. The mean times to death for the control populations were used to represent the foundation generations since neither mean time to death nor σ_p (before selection) could be calculated without killing an entire foundation generation.

The validity of the results depended on the homogeneity of treatment environments (and pre-treatment environments) of both resistant and susceptible (control) F_1 groups. Therefore, treatment of both offspring groups was performed in a plastic container divided

into two equal compartments by a nylon screen.

Seven realized heritability estimates, corresponding to seven groups of resistant, F_1 progeny and their controls, were determined from the two mass selection experiments. Five separate groups of F_1 progeny were obtained from the resistant parents initially selected from foundation generation 1. The groups were treated by the standing water bioassay method at concentrations ranging from 5 to 8 mg/l Pb to determine if any correlation exists between heritability estimates and treatment concentrations. The two groups of F_1 progeny obtained from parents selected out of foundation generation 2 were treated at 15 mg/l Pb (S.W.B.).

The Uptake of Lead From Contaminated Solutions

To determine the levels of lead concentrated following exposure to contaminated solutions, test populations were treated at concentrations that ranged from 5 - 20 mg/l Pb in standing water bioassays and 10 mg/l Pb in flowing water bioassays. Analyses for concentrated levels of lead were performed with a Varian Techtron, Model 1200, Atomic Absorption Spectrophotometer. Use of the unit's "Concentration Mode" enabled values to be read directly in parts per million lead (μg/gm or mg/l Pb). The hollow cathode lead lamp was operated at 5.0 mA, slit width was 1.0 nm and wavelength was approximately 217 nm with sensitivities to 0.13 mg/l Pb. An

air-acetylene flame was used. Any fish tissues to be analyzed were sprayed lightly with distilled water and blotted with paper towels prior to weight measurements. Tissue samples were ashed in porcelain crucibles at 500°C for approximately 18 hours. The ash was then dissolved in 1 N HCl and analyzed for lead content. Standard lead solutions provided a basis for the determination of the unknown samples.

Increased Resistance

Four experiments were performed to determine the relationship between increased resistance and the amount of lead concentrated in the body. The lead resistant parents initially selected from foundation generation 1 and a previously unselected control group (both 10 months of age) were treated in the first experiment. This provided for a comparison of the relationship between increased resistance and the amount of lead concentrated in the fish of two "different" populations. The treatment consisted of a flowing water bioassay at 10 mg/l Pb for 7.5 hours. (The "sublethal" exposure concentrations and time periods used in these studies were estimated from preliminary bioassays.) Both populations were treated simultaneously in a plastic container which was divided into two compartments by a nylon screen. Upon initiation of the experiment, subsamples of five fish each were removed from the two treatment

populations to be analyzed for whole body burden of lead. Subsamples were also removed throughout the exposure period. At the end of 7.5 hours, the remainder of each fish population was removed from the treatment solution and placed into separate containers. The containers were provided with a constant flow of dechlorinated water at room temperature (22°C). Subsampling was continued for two weeks after which analyses were performed for whole body burden of lead.

A second experiment was performed with an additional resistant population and a control. These fish were one year in age. Treatment conditions were similar to those used above except that the two groups were treated for 8.5 hours.

The third study was performed with offspring (age 2 months) obtained prior to treatment of the populations used in the first experiment. These offspring groups were treated for 7.0 hours under conditions similar to those used above.

The fourth treatment was performed on a second group of offspring that were ten days old. These, and a control were treated at 5 mg/l Pb for 6.5 hours.

RESULTS AND INTERPRETATION

Heritability Estimates

The first objective of this study was to determine the heritability of resistance to the effects of lead. Analysis of the variability in time to death between sibling groups of guppies permitted estimation of the variance components associated with each source of variation (Comstock and Robinson, 1952; Becker, 1967). Results of the analysis of variance in time to death for fish in experiments A and B are presented in Tables 1 and 2, respectively.

Table 1. Analysis of variance in time to death for sib analysis A.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Between males	15	9,982	665.5	2.77*
Between females within males	16	3,840	240.0	2.11*
Progeny within females	288	32,418	112.6	

Components of variance:

$$\sigma^2_M = 21.25;\ \sigma^2_F = 12.7;\ \sigma^2_W = 112.6$$

*Statistical significance at the 0.05 level.

Table 2. Analysis of variance in time to death for sib analysis B.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Between males	7	5,755	822	4.09*
Between females within males	8	1,604	201	
Progeny within females	304	82,724	272	
Components of variance:				
$\sigma^2_M = 15.54$; $\sigma^2_F = 0$; $\sigma^2_W = 272$				

*Statistical significance at the 0.05 level.

For the first experiment (A), the variance component estimated for males was 21.25; therefore, V_A, which was estimated as $4\sigma^2_M$ was equal to 85.0. The total phenotypic variance ($V_P = \sigma^2_M + \sigma^2_F + \sigma^2_W$) was 146.95. Heritability (V_A/V_P) of resistance to the toxic effects of lead under the conditions used in this study was 0.58 with a standard error (Becker, 1967) of 0.32. The heritability estimated from results of experiment B was 0.26 with a standard error of 0.17. The absence of any variance component that could be attributed to differences between females in experiment B was most likely an aberrant result arising from the low numbers of male and female parents.

The breeding design used in this study had the potential of generating five, half-sibling families for each of the twenty male

parents. This potential was never realized since sterility and other factors may have limited the numbers of families obtained. In addition, the females did not all spawn at the same time. If families of offspring were not treated at similar ages, weight or other age-related differences could affect any experimental results. Consequently, the groups of offspring tested in this study represent a biased sample of the potential numbers available and the variances may be underestimated. Finally, precise times to death for all the individuals tested were not recorded. The resulting class effect may have influenced the residual component of variance, σ^2_W.

The results of mass selection experiments that were performed to determine the actual, or realized, response to selection for increased resistance to lead are presented in Tables 3 and 4. Herein are the responses determined from several offspring groups of two parental populations selected for increased resistance from foundation generations 1 and 2, respectively. The response to treatment of the seven groups of resistant F_1 progeny, when compared to seven susceptible (control) progeny groups, permitted the determination of the heritability estimates. These estimates ranged from 0.288 to 0.679 and are of comparable magnitude to the heritabilities determined by the nested mating experiment. There was no apparent correlation between the heritability estimates and the treatment levels.

Table 3. Summary of heritability estimates (h^2) for lead resistance from foundation generation 1. Approximately 18 percent were selected as resistant progenitors of the F_1 groups listed. Response differences between resistant and susceptible (control) F_1 progeny yielded h^2. Numbers treated are in parentheses.

Generation	Treatment level (mg/1 Pb)	Standard deviation (σ_P)	Mean time to death (hrs.)	Selection intensity (i)	Response (R) in hours	Heritability (h^2)	Standard error (Prout, 1962)
Foundation 1 (1700)	5	Estimated from		1.475			
Control (300)	0	F_1 controls					
F_1 resistant (64)	5	20.57	50.20		11.45	0.314	0.064
F_1 control (64)	5	24.69	38.75				
F_1 resistant (66)	5	21.71	55.31		17.62	0.433	0.068
F_1 control (66)	5	27.60	37.69				
F_1 resistant (100)	6	22.03	47.10		15.12	0.666	0.097
F_1 control (100)	6	15.39	31.98		15.43	0.679	0.090
F_1 resistant (100)	6	20.26	47.41				
F_1 resistant (100)	8	8.99	23.32		4.96	0.449	0.082
F_1 control (100)	8	7.48	18.36				

Table 4. Summary of heritability estimates (h^2) for lead resistance from foundation generation 2. Approximately 22 percent were selected as resistant progenitors of the F_1 groups listed. Response differences between resistant and susceptible (control) F_1 progeny yielded h^2. Numbers treated are in parentheses.

Generation	Treatment level (mg/l Pb)	Standard deviation (σ_P)	Mean time to death (hrs.)	Selection intensity (i)	Response (R) in hours	Heritability (h^2)	Standard error (Prout, 1962)
Foundation 2 (1000)	15	Estimated from F_1 controls		1.348			
Control (200)	0						
F_1 resistant (200)	15	15.61	36.33		9.79	0.613	0.073
F_1 control (200)	15	11.86	19.80				
F_1 resistant (200)	15	12.41	32.44		4.24	0.288	0.062
F_1 control (200)	15	10.99	28.20				

The Uptake of Lead From Contaminated Solutions

To determine the levels of lead which may have been concentrated by test fish in the selection experiments described, similar studies were done on smaller groups of experimental guppies. Atomic Absorption Spectrophotometry was used to detect the levels of lead concentrated in various tissues (Tables 5 and 6). In every case the head area concentrated the highest levels of lead; appreciable amounts were also detected in the viscera. In the present study, trails of mucus were frequently observed about the head region. Increased opercular movements were apparent. The stress observed could have been caused by "coagulation film anoxia" (Westfall, 1945; Jones, 1964). It is possible that exposed fish are binding lead in the head (gill) mucus and attempting to "slough off" this bulky layer. Copious mucus producers might be able to survive the effects of lead for longer time periods (Table 6).

Increased Resistance

Rates of uptake and elimination of lead were compared between a previously selected, Pb resistant population, and an unselected control group. Both groups were ten months of age. Analyses performed on the tissues from fish in these groups indicated that resistant fish concentrated higher amounts of lead more rapidly

Table 5. Mean amounts of lead concentrated (μg/gm) in the heads and body tissues of ten killed guppy fry. The individuals were treated at 5 mg/l Pb in three liters for 12 hours in a standing bioassay. Numbers treated are in parentheses.

	Head Portion		Remaining Body Tissues	
Container	Wet weight (gms)	Pb detected (μg/gm)	Wet weight (gms)	Pb detected (μg/gm)
1 (5)	0.0602	108.30	0.0432	56.20
2 (5)	0.0443	91.42	0.0352	60.40

Table 6. Levels of lead in various tissues following treatment at 10 and 20 mg/l Pb (standing) and 10 mg/l Pb (flowing bioassay). Numbers treated are in parentheses. (These represent random subsamples from larger populations.)

Treatment solution		Wet weight (gms) of surviving fish (3)	Concentrated Pb (μg/gm)	Wet weight (gms) of susceptible fish (3)	Concentrated Pb (μg/gm)
10 mg/l (standing bioassay for 24 hours)	liver	0.056	30.0	0.055	25.11
	flesh	0.507	2.0	0.460	5.40
	skin	0.177	5.85	0.147	35.70
	head	0.460	32.60	0.529	67.36
20 mg/l (standing bioassay for 24 hours)	liver	0.042	37.50	0.047	62.50
	flesh	0.431	5.80	0.571	13.15
	skin	0.127	62.50	0.153	50.00
	head	0.338	63.60	0.534	74.90

	Survivors (5)		Susceptible fish (15)	
	Mean amount Pb (μg/gm) concentrated in viscera	Mean amount Pb (μg/gm) concentrated in remaining body tissues	Mean amount Pb (μg/gm) concentrated in viscera	Mean amount Pb (μg/gm) concentrated in remaining body tissues
10 mg/l (flowing bioassay for 18 hours)	38.98	55.85	51.65	65.18

than did control fish. Upon transfer to an uncontaminated, flowing water supply, a sharp decline in whole body burden of lead was detected in the resistant group (Figure 2). This suggests that resistant fish eliminated lead at a greater rate than did the control group.

A second experiment was performed with an additional resistant population and a control group. Both were treated at the age of one year. Results of this study confirmed the results of the first experiment (Figure 3).

Two additional experiments involved the treatment of F_1 progeny from the resistant and control populations tested in the first experiment. Although the differences in rates of lead uptake between resistant and control adults were maintained in their offspring, the rates of elimination were inconsistent (Figures 4 and 5). No explanation for the differences between elimination rates of the resistant offspring groups was evident. Since differences in uptake rates between resistant and control groups of adults were maintained in their progeny (Figures 2 - 5), a genetic basis for these differences was implied.

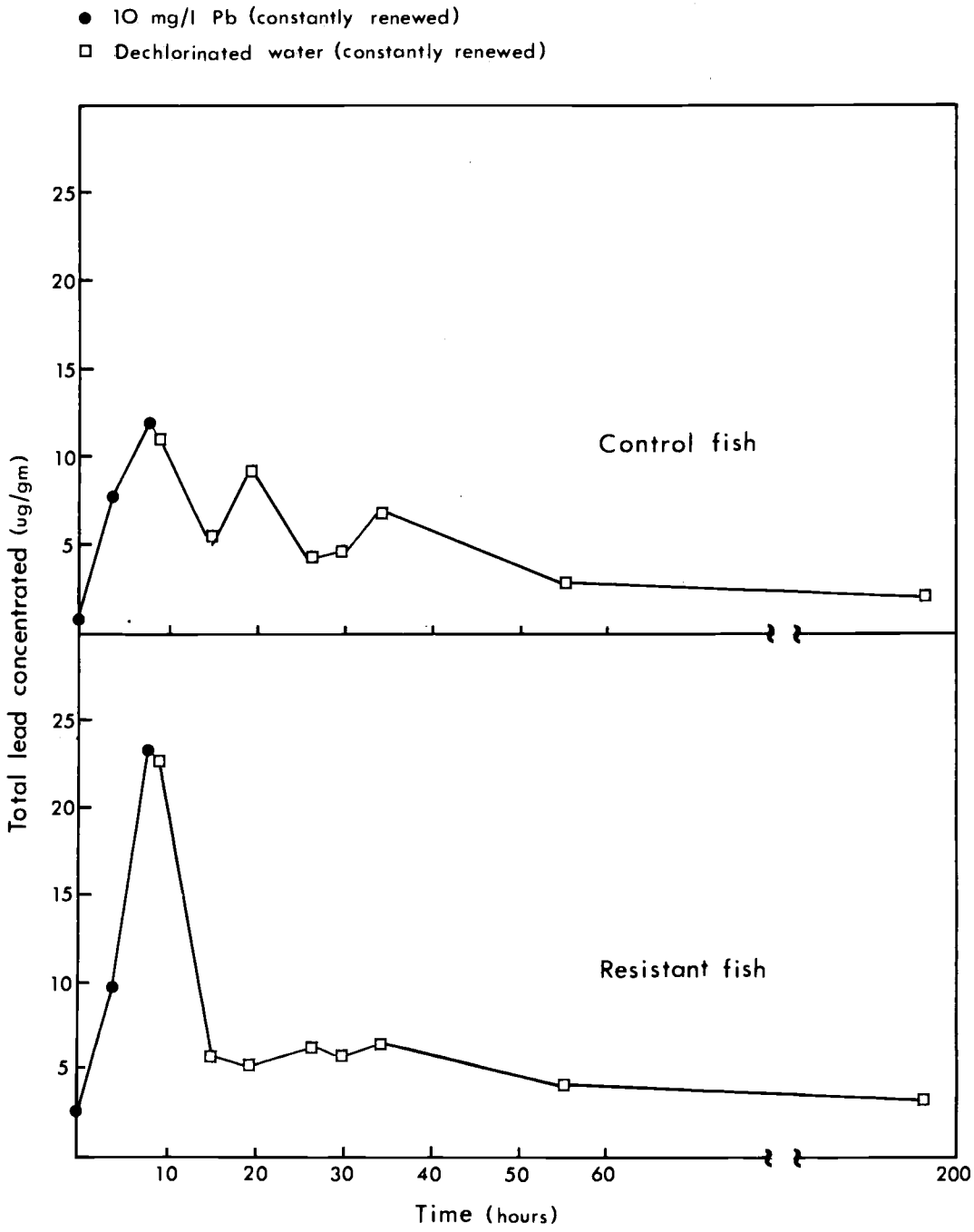

Figure 2. Lead uptake and loss by two experimental populations of guppies, ten months of age. Both groups were exposed to 10 mg/l Pb for 7.5 hours. (Each point represents μg/gm Pb concentrated by five fish.)

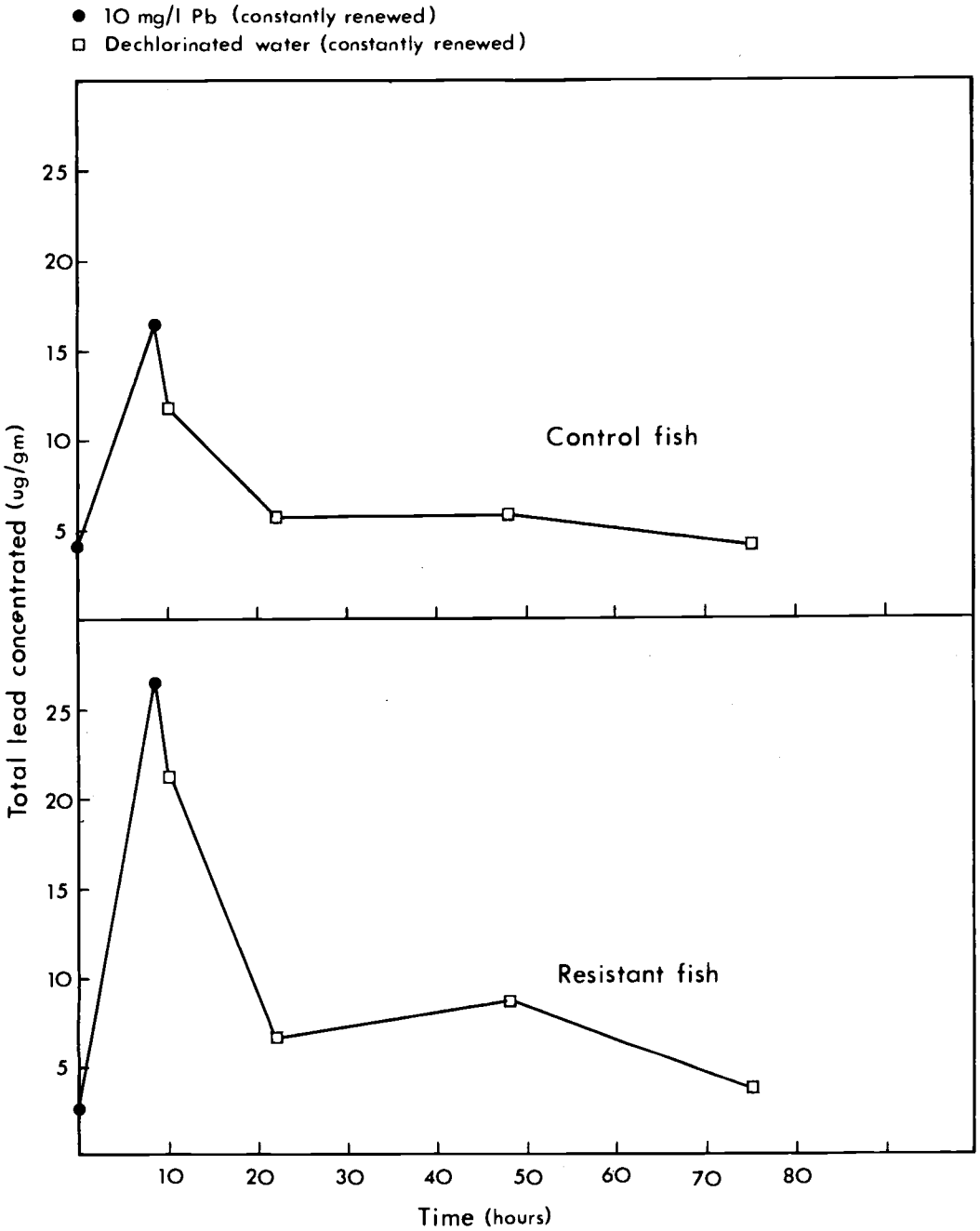

Figure 3. Lead uptake and loss by two experimental populations of guppies, one year of age. Both groups were exposed to 10 mg/l Pb for 8.5 hours. (Each point represents µg/gm Pb concentrated by five fish.)

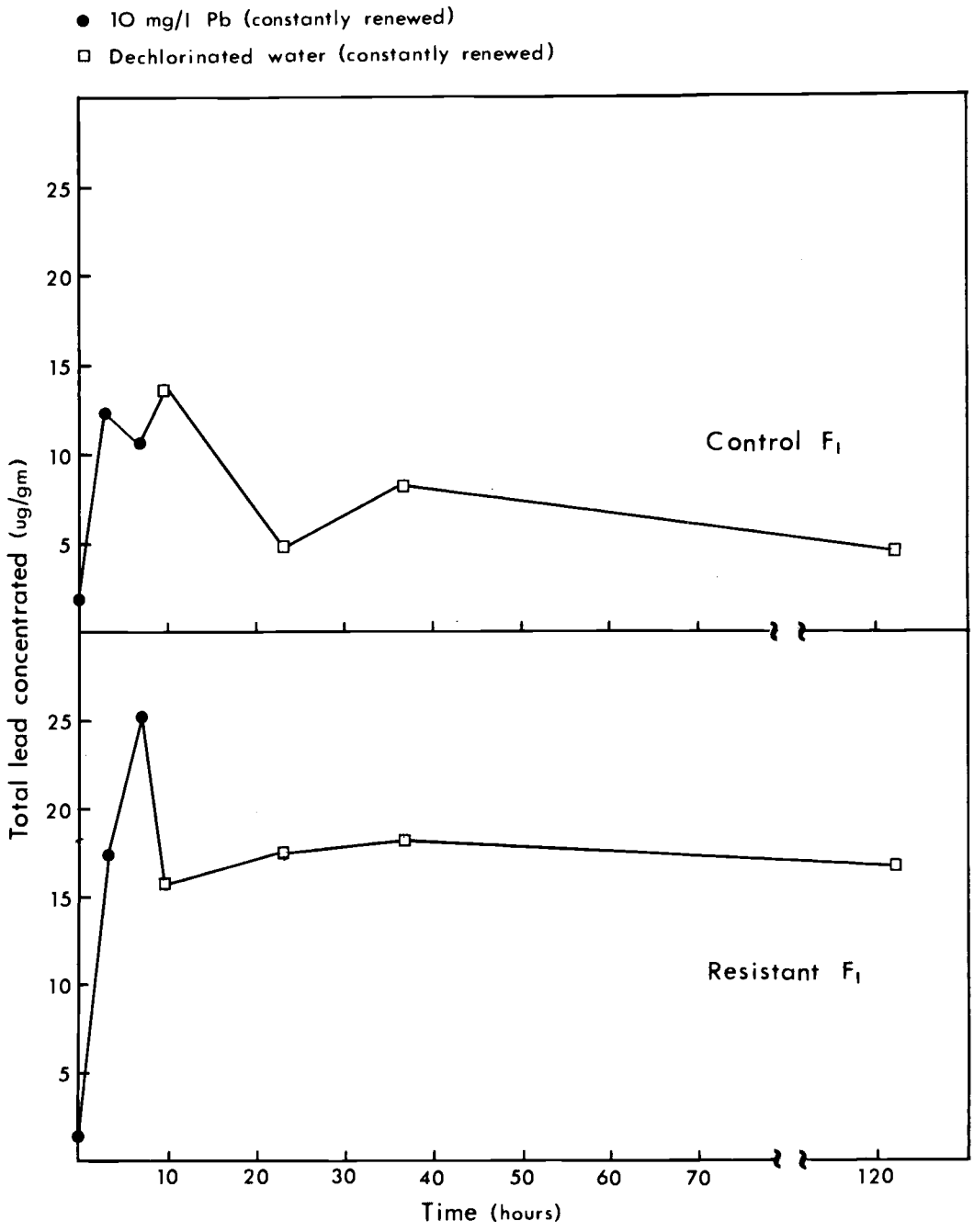

Figure 4. Lead uptake and loss by two experimental populations of guppies (F_1) at two months of age. Both groups were exposed to 10 mg/l Pb for 7.0 hours. (Each point represents μg/gm Pb concentrated by five fish.)

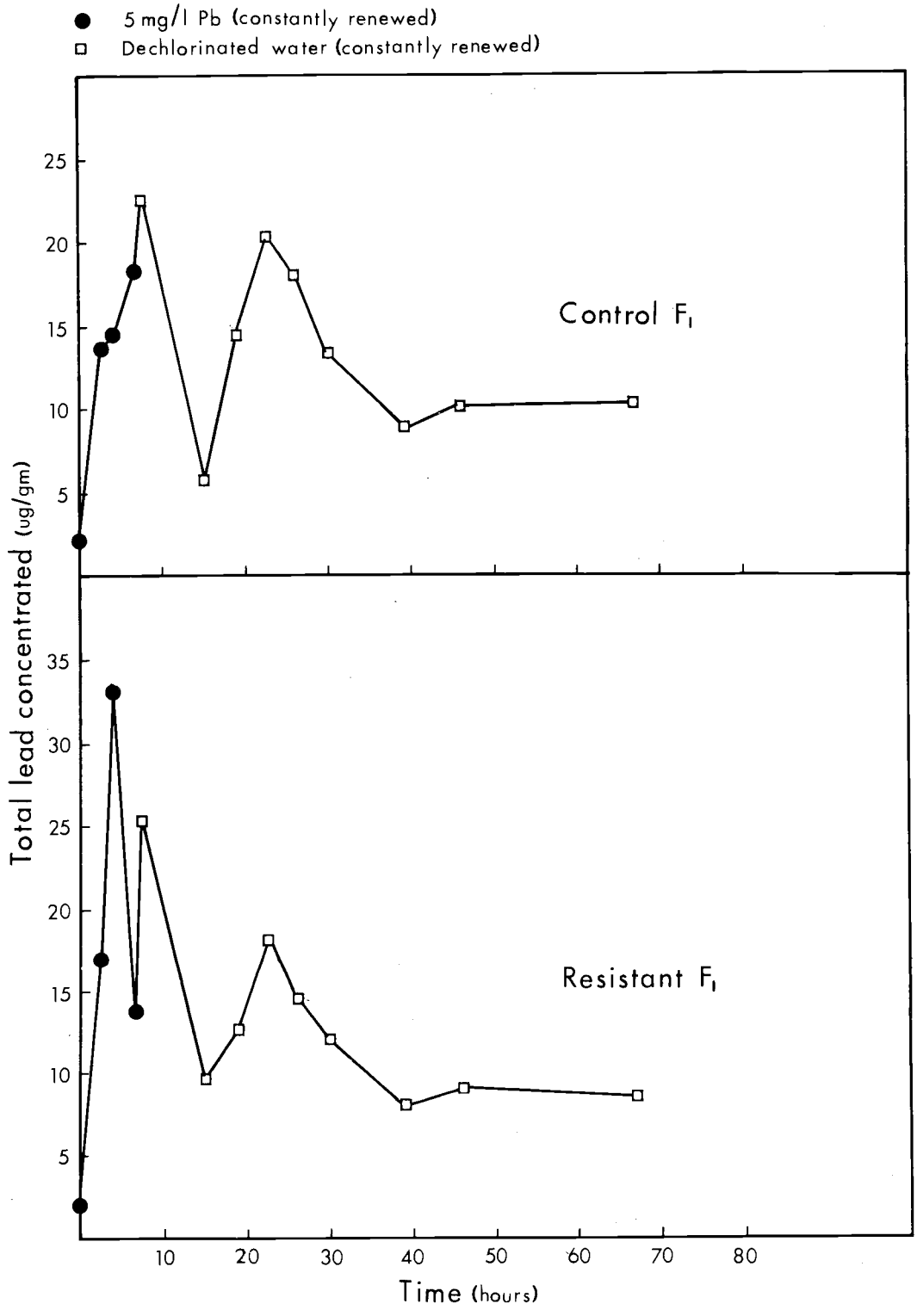

Figure 5. Lead uptake and loss by two experimental populations of guppies (F_1) at ten days of age. Both groups were exposed to 5 mg/l Pb for 6.5 hours. (Each point represents μg/gm Pb concentrated by five fish.)

DISCUSSION

Physiological differences (related to age of experimental fish) and environmental differences existed between experiments. Accordingly, a range of heritability estimates was determined in this study. Heritabilities of resistance to lead from sib comparisons were estimated to be 0.26 and 0.57. Realized heritability estimates ranged from 0.28 to 0.68. The results of these experiments suggest that valid estimates of heritability can be obtained by mating experiments.

The genetic implication of these experiments is that resistance, measured here by mean time to death, could be expected to increase in fish populations experiencing lead-related mortality. Significant amounts of lead are found in areas adjacent to heavily travelled roadways and certain industries (Cooper, 1970; McIntire and Angle, 1972; Smith, 1971).

If increased resistance to heavy metals involves greater concentrations in fish tissues, a threat to predator organisms may exist. Uptake rates of lead were greater in resistant guppies than in control groups. In previous uptake studies, presence of greater amounts of lead in head regions was demonstrated. This may be the result of the binding of lead to macromolecular components of the gill mucus. The sharp rise in lead content during treatment of

the resistant groups might be attributed to efficient binding of lead in this gill mucus. Following transfer of experimental fish to an uncontaminated water supply, elimination of lead that was concentrated during treatment was observed in three resistant groups. If resistant fish are copious mucus producers, these mucus layers about the gills may be "sloughed off" at greater rates. Data are not available to explain why one of the resistant populations concentrated lead for an extended time period. More information concerning the role of gill mucus in resistance to lead toxicity is required.

CONCLUSIONS

1. The variation in resistance to death from the effects of lead in laboratory populations of guppies was observed to have a significant genetic component.

2. The results of mass selection experiments indicate that valid estimates of heritability can also be obtained from breeding experiments of limited size.

3. Lead was concentrated in the head region to a greater extent than in other body tissues.

4. Lead resistant fish concentrated lead more rapidly than control groups. A genetic basis for this difference was implied.

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APPENDICES

APPENDIX I

Characteristics of the Water Supply Used

Type	Dissolved oxygen	Alkalinity	Hardness	pH	Electrical conductivity
Dechlorinated tap water	10.3 mg/l	33.5 mg/l $CaCO_3$	28.0 mg/l $CaCO_3$	6.8	90 μmhos/cm

APPENDIX II

Analysis of Variance and the Estimation of Heritability From a Nested Breeding Design

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Expected mean squares
Between males	$m - 1$	SS_m	MS_m	$\sigma^2_w + k_2\sigma^2_f + k_3\sigma^2_m$
Between females within males	$f - m$	SS_f	MS_f	$\sigma^2_w + k_1\sigma^2_f$
Progeny within females	$n... - f$	SS_w	MS_w	σ^2_w

where: m = number of males
f = number of females
$n...$ = total number of progeny

with equal numbers of females/male and progeny/female:
$k_1 = k_2$ = number of progeny/female
k_3 = number of progeny/male

Variance component			Interpretation
σ^2_m	=	$\frac{MS_m - MS_f}{k_3}$	1/4 additive genetic variance + paternal effects.
σ^2_f	=	$\frac{MS_f - MS_w}{k_1}$	1/4 additive genetic variance + maternal effects.
σ^2_w	=	MS_w	Remainder of genetic variance + environmental variance + experimental error.

Heritability Estimation:

Based on males, $h^2 = \frac{4\,\sigma^2_m}{\sigma^2_m + \sigma^2_f + \sigma^2_w} = \frac{V_A}{V_P}$