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# Hepatic Glutathione *S*-Transferase Activity in Mice: Effects of $A^{vy}/-$ Genotype, Obesity, Lindane Treatment, and Sex (42289)

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*Abstract.* Phenotypically distinct but genetically identical obese mottled yellow $A^{vy}/a$ and lean pseudoagouti $A^{vy}/a$ sibling mice and their congeneic black $a/a$ littermates provide an experimental system for distinguishing phenotypic effects from genotypic effects in the expression of the genotype at the organismic level. Hepatic glutathione *S*-transferase activity in obese yellow $A^{vy}/a$ (YS × VY) F-1 hybrid female mice was only about 66% of that found in their lean black $a/a$ sisters. This decreased enzyme activity was not a direct effect of the $A^{vy}/a$ genotype but was associated with the obesity of the yellow mice since the enzyme activity in lean pseudoagouti $A^{vy}/a$ female siblings was similar to that found in the black $a/a$ mice. Long-term feeding of 160 ppm lindane in the diet decreased the enzyme activity in all phenotypes but did not eliminate the difference between the obese yellow and lean pseudoagouti and black mice. Interpretation of the available data suggests that no direct relationship exists between the level of hepatic glutathione *S*-transferase activity and the enhancement of tumor formation in yellow $A^{vy}/a$ mice. Several inbred mouse strains and F-1 hybrids were also screened for this enzyme activity. No strain differences were found but sex differences within different inbred strains were not uniform. In the AE and YS strains and their F-1 hybrid enzyme activity was higher in females than in males. In contrast, BALB/c and VY strain males had higher enzyme activity than the corresponding females.

Obesity, enhancement of hyperplasia and neoplasia, and dysregulation of hair pigment pattern are major phenotypic manifestations of the $A^{vy}$ (viable yellow) mutation at the agouti locus in the house mouse, *Mus musculus*. This locus exerts its influence on the regulation of cellular metabolism through modulation of unknown conditions in the tissue in which the cells expressing the phenotypic effects are located. This has been demonstrated for melanin synthesis (1), excess fat deposition (2), and more rapid growth of neoplastic cells (3) in yellow $A^{vy}/a$ and $A^{y}/a$ mice. Decreased latent period and increased prevalence characterize the increased susceptibility of yellow mice to virally and chemically induced hyperplasia and neoplasia (4–6).

In a previous study (6) we observed that obese yellow mice had somewhat lower hepatic concentrations of reduced glutathione (GSH) than their lean nonyellow littermates. This suggested that decreased detoxification of toxicants or their carcinogenic metabolites by conjugation with GSH might contribute to the increased tumor prevalence in these mice.

The enzyme glutathione *S*-transferase (GST) catalyzes the conjugation of toxicants with GSH as the first step in the metabolic pathway leading to formation and excretion of mercapturic acid (7). To determine whether this enzyme activity might be decreased in the more tumor-susceptible yellow mice, the activity of hepatic GST with the substrate 1-chloro-2,4-dinitrobenzene was assayed. This was done as part of a chronic study to ascertain differential susceptibility of female obese yellow $A^{vy}/a$, lean pseudoagouti $A^{vy}/a$, and lean black $a/a$ (YS × VY) F-1 hybrid mice to tumor formation in response to exposure to lindane (γ-hexachlorocyclohexane). This enzyme activity did prove to be lower in these yellow mice than in the genetically identical or congeneic nonyellow mice; however, the pseudoagouti and black mice had similar enzyme activity levels but differed significantly in susceptibility to lindane-induced enhancement of liver and lung tumor formation (8). Therefore, there appears to be no direct correlation between the level of activity of this enzyme and

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the different degrees of tumor susceptibility of the three mouse phenotypes.

Since we did find significant differences in enzyme activity between the yellow and non-yellow (YS × VY) F-1 hybrid mice, GST activity and hepatic glutathione levels were also measured in other F-1 hybrids and inbred strains. We wished to determine whether the differences found among (YS × VY) F-1 mice were dependent on the particular genetic background and also whether other common inbred strains might differ significantly from each other in GST activity.

**Materials and Methods.** *Animals.* For the lindane study female mottled yellow $A^{vy}/a$, pseudoagouti $A^{vy}/a$, and black $a/a$ (YS × VY) F-1 hybrid mice were produced in the NCTR breeding colony in a specific pathogen free (SPF) barrier-sustained environment (9) by mating inbred black $a/a$ YS/ChWffC3Hf/Nctr dams by inbred yellow and pseudoagouti $A^{vy}/a$ VY/WffC3Hf/Nctr sires. The mice were allocated to the study at weaning. They were housed three per cage and maintained in a barrier-sustained environment. Feeding of sterilized Purina Laboratory Chow 5010M containing a target dose of 160 ppm lindane or untreated feed began at about 4 weeks of age and continued until sacrifice of the mice at 13–14 or 18–19 months of age. Feed and filtered water, which had been heated to about 200°F and then cooled to ambient temperature, were always available.

For the screening study mice of the following inbred strains and F-1 hybrids were also produced in the NCTR breeding colony: AM/WffC3Hf/Nctr-$A^{vy}$,$a^{m}$ (AM), AE/WffC3Hf/Nctr (AE), BALB/cStCrlfC3Hf/Nctr (BALB/c), C57BL/6JfC3Hf/Nctr (C57BL/6J), C57BL/6NfC3Hf/Nctr (C57BL/6N), C3H/HeN-MTV-/Nctr (C3H-MTV-), VY/WffC3Hf/Nctr-$A^{vy}$ (VY),YS/ChWffC3Hf/Nctr-$A^{vy}$ (YS), (AE × YS)$F_1$, (BALB/c × VY-$A^{vy}$)$F_1$, (C57BL/6N × C3H-MTV-)$F_1$. They were held to 60 days of age in a clean, conventional animal room. Four mice of the same sex were housed in each 6.5 × 11.5-in. polycarbonate cage covered with a molded filter bonnet of spun bonded polyester. Purina Laboratory Chow 5010C and filtered water were always available. Sterilized hardwood chips (Sani-Chips, P. J. Murphy Forest Products Corporation, Rochelle Park, N.J.) were used as bedding. Cages, feed, and water bottles were changed weekly. Room temperature was maintained at 22 ± 2°C and relative humidity at 50 ± 9% with 15 changes of air per hour. Light cycles of 12 hr light: 12 hr dark with no twilight intervals were automatically regulated and began at 6 AM.

*Biochemical assays.* Nonfasted mice were killed by $CO_2$ inhalation between 9 and 10 AM and the livers removed. Each liver was divided into two approximately equal portions, weighed, and homogenized with a Polytron (Brinkman Instruments, Westburg, N.Y.) in ice-cold (4°C) 0.1 *M* phosphate buffer, pH 6.5. The homogenate was centrifuged at 10,000*g* for 30 min in a refrigerated (5°C) centrifuge (CRU-5000, International Equipment Company, Needham Heights, Mass.). The supernatant was then centrifuged in an L2-65B ultracentrifuge (Beckman Instruments, Palo Alto, Calif.) at 100,000*g* for 30 min, 5°C. The supernatant was removed and immediately analyzed for reduced glutathione (GSH) and glutathione *S*-transferase (GST).

Reduced glutathione was proportional to the reduction of 5,5′-dithiobis(2-nitrobenzoic acid at 412 nm, 30°C (10, 11). Five m*M* GSH (Sigma Chemical Co., St. Louis, Mo.) was used as a standard.

GST activity was determined with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [(7) B Ketterer, personal communication]. The kinetic assays were conducted with a spectrophotometer (Model 100-80A, Hitachi Instruments, Mountain View, Calif.) with a constant temperature cell holder at 340 nm, 37°C. The molar extinction coefficient was 9.6 $mM^{-1}$ $cm^{-1}$. Glutathione *S*-transferase (Sigma Chemical) served as a positive control for daily analyses. Cytosolic protein was determined by a biuret method (Worthington Diagnostics, Freehold, N.J.). The activity measured represents the sum of activities of several isozymes with overlapping substrate specificities and different relative substrate preferences (AM Benson, personal communication).

Assay data were grouped to form replicates according to assay date. As appropriate, analyses of variance were conducted using sex, genotype, strain, and assay date. Differences between sexes within strains were compared by

paired $t$ tests. Differences between strains within sexes were compared by $t$ tests and adjusted for multiple comparisons by the Studentized Range (12).

**Results.** As briefly reported previously (13), hepatic glutathione S-transferase activity was significantly ($P = 0.01$) lower in obese yellow $A^{vy}/a$ mice than in their genetically identical lean pseudoagouti $A^{vy}/a$ and lean black $a/a$ littermates (Table I). This difference in enzyme activity between the obese and lean mice was also present in lindane-fed mice even though the lindane treatment itself decreased the enzyme activity in all of the phenotypes approximately to the same degree. In contrast to their effects on GST activity, neither phenotype nor lindane feeding appeared to affect the concentration of reduced glutathione in the liver.

Liver weights of obese yellow mice were significantly greater ($P = 0.01$) than those of pseudoagouti and black mice in line with previous studies (14). Lindane feeding increased liver weight ($P = 0.005$) in all mouse phenotypes in conformity with previous reports (15).

When yellow and nonyellow mice of the inbred strains and F-1 hybrids were compared with each other at 2 months of age (Table II), the lower enzyme activity in the yellow female mice was confirmed. In general, the hepatic enzyme activity in yellow females was about two-thirds that in the nonyellow littermates (Table III). Among the males, there was no uniformity in enzyme activity differences between yellow and nonyellow littermates among the different genetic stocks (Table II).

Sex differences in enzyme activity in nonyellow mice fell into two groups. In one group, comprised of the inbred AE and YS strains and their F-1 hybrid, females had greater enzyme activity than the males (Tables II and

TABLE I. EFFECT OF $A^{vy}/a$ PHENOTYPES AND CHRONIC LINDANE TREATMENT ON HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY, REDUCED GLUTATHIONE CONCENTRATION, AND LIVER WEIGHT IN (YS × VY) F-1 HYBRID FEMALE MICE

| Phenotype: | Obese yellow | Lean pseudoagouti | Lean black |
|---|---|---|---|
| Genotype: | $A^{vy}/a$ | $A^{vy}/a$ | $a/a$ |
| | Mean ± SE ($n$) | Mean ± SE ($n$) | Mean ± SE ($n$) |
| | Hepatic glutathione S-transferase activity[a] (μm/min/mg total protein) | | |
| Control[b] | 1415 ± 272 (12)[f] | 2844 ± 547 (12) | 3618 ± 696 (12) |
| Control[c] | 1042 ± 504 (9)[f] | 2448 ± 369 (12) | 2481 ± 575 (14) |
| Lindane[b,d] | 779 ± 184 (9)[f] | 1183 ± 227 (12) | 1380 ± 291 (11) |
| Lindane[c,e] | 304 ± 61 (12)[f] | 772 ± 94 (13) | 1341 ± 298 (13) |
| | Hepatic reduced glutathione (μg/mg total protein) | | |
| Control[b] | 6.38 ± 0.60 (12) | 6.29 ± 0.59 (12) | 7.37 ± 0.69 (12) |
| Control[c] | 2.69 ± 0.70 (9) | 3.77 ± 0.59 (12) | 4.89 ± 0.56 (14) |
| Lindane[b,d] | 4.72 ± 0.48 (12) | 5.59 ± 0.52 (12) | 6.26 ± 0.64 (12) |
| Lindane[c,e] | 2.68 ± 0.60 (12) | 2.85 ± 0.57 (13) | 4.36 ± 0.57 (13) |
| | Liver weights (g) | | |
| Control[b] | 2.24 ± 0.10 (12) | 1.60 ± 0.05 (12) | 1.55 ± 0.04 (12) |
| Control[c] | 2.86 ± 0.39 (9) | 1.58 ± 0.03 (12) | 1.74 ± 0.10 (14) |
| Lindane[b,d] | 2.98 ± 0.11 (12) | 1.84 ± 0.08 (12) | 1.75 ± 0.06 (12) |
| Lindane[c,e] | 3.28 ± 0.12 (12) | 2.08 ± 0.08 (13) | 1.93 ± 0.06 (13) |

[a] Substrate: 1-chloro-2,4-dinitrobenzene.
[b] 13–14 months old.
[c] 18–19 months old.
[d] Fed 160 ppm lindane in feed for 12–13 months beginning at 1 month of age.
[e] Fed 160 ppm lindane in feed for 17–18 months beginning at 1 month of age.
[f] Significantly ($P = 0.01$) lower than the pseudoagouti and black mice.

TABLE II. HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY (SUBSTRATE: 1-CHLORO-2,4-DINITROBENZENE) (μm/min/mg TOTAL PROTEIN)

| Strain | Obese yellow $A^{vy}/-$ mice | | Lean nonyellow mice | |
|---|---|---|---|---|
| | Male Mean ± SE (*n*) | Female Mean ± SE (*n*) | Male Mean ± SE (*n*) | Female Mean ± SE (*n*) |
| AM | ND[a] | 2280 ± 232 (12) | ND | 3801 ± 468 (12) |
| AE | NA[b] | NA | 766 ± 104 (12) | 2163 ± 279 (12) |
| YS[c] | ND | 2320 ± 240 (11) | ND | 2030 ± 210 (11) |
| | 1295 ± 500 (3) | 1677 ± 189 (11) | 942 ± 147 (8) | 2496 ± 332 (11) |
| | 776 ± 81 (14) | 1415 ± 142 (15) | 466 ± 69 (14) | 2280 ± 342 (15) |
| (AE × YS)$F_1$ | NA | NA | 508 ± 128 (12) | 1816 ± 113 (15) |
| BALB/c[c] | NA | NA | ND | 2559 ± 774 (11) |
| | NA | NA | 5930 ± 503 (16) | 1055 ± 112 (15) |
| VY[c] | ND | 1389 ± 326 (12) | ND | 2896 ± 550 (9) |
| | 4317 ± 401 (5) | 756 ± 97 (15) | 5642 ± 566 (5) | 1555 ± 211 (15) |
| (BALB/c × VY)$F_1$ | 1300 ± 210 (15) | 1590 ± 240 (16) | 1670 ± 360 (15) | 2470 ± 570 (15) |
| C57BL/6J | NA | NA | ND | 2750 ± 210 (11) |
| C57BL/6N | NA | NA | ND | 3520 ± 420 (11) |
| C3H-MTV- | NA | NA | ND | 2350 ± 728 (12) |
| (C57BL/6N × C3H-MTV-)$F_1$ | NA | NA | ND | 3420 ± 430 (11) |

[a] Not determined.
[b] Not applicable—no yellow mice produced.
[c] Each horizontal line of data represents a different set of mice at 8 weeks of age. Sets were assayed several months apart.

IV). In contrast, in the inbred BALB/c and VY strains, males exhibited greater enzyme activity than the females (Tables II and IV).

Based on comparison of the data from nonyellow females, there appear to be no consistent differences in enzyme activity among the different inbred strains and F-1 hybrids. There are, however, considerable differences in enzyme activity levels within at least the BALB/c and VY strain mice when assays performed at the same time of day but several months apart were compared (Table II).

**Discussion.** The $A^{vy}/a$ mouse genotype is expressed in two phenotypic classes, obese mottled yellow and lean pseudoagouti. Pseudoagouti mice cannot be distinguished visually from agouti $A/A$ mice. In $A/A$ mice the hairs are black or brown with a subterminal yellow band resulting from the cyclical synthesis of two ultrastructurally different types of melanosomes by the endoplasmic reticulum of the

TABLE III. RATIO OF HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY (SUBSTRATE: 1-CHLORO-2,4-DINITROBENZENE) IN YELLOW MICE TO THAT IN NONYELLOW SIBLINGS

| Sex | Background genome | Yellow/ nonyellow |
|---|---|---|
| Male | VY | 0.765 |
| | YS | 1.665 |
| | YS | 1.375 |
| | BALB/c × VY | 0.778 |
| | Mean ratio | 1.146 |
| Female | AM | 0.600 |
| | VY | 0.486 |
| | VY | 0.480 |
| | YS | 0.621 |
| | YS | 0.679 |
| | YS | 1.143 |
| | BALB/c × VY | 0.644 |
| | Mean ratio | 0.665[a] |

[a] $P < 0.01$.

TABLE IV. DIRECTION OF GENOTYPE AND SEX DIFFERENCES IN HEPATIC GLUTATHIONE *S*-TRANSFERASE ACTIVITY (DATA IN TABLE II)

| Strain | Replicate No. | Male | Female | Female cf. Male | *P* |
|---|---|---|---|---|---|
| AM | | | N[a] > Y[b] | | 0.05 |
| AE | | | | $F_N$[c] > $M_N$[d] | <0.01 |
| YS | 1 | | N = Y | | >0.05 |
| | 2 | | N > Y | | 0.01 |
| | | N = Y | | | >0.05 |
| | | | | $F_Y = M_Y$ | 0.06 |
| | | | | $F_N > M_N$ | 0.01 |
| | 3 | | N > Y | | 0.03 |
| | | N < Y | | | 0.007 |
| | | | | $F_Y > M_Y$ | 0.00008 |
| | | | | $F_N > M_N$ | 0.0001 |
| AE × YS | | | | $F_N > M_N$ | <0.003 |
| BALB/c | | | | $F_N < M_N$ | 0.0001 |
| VY | 1 | | N > Y | | <0.05 |
| | 2 | N = Y | | | >0.05 |
| | | | N > Y | | 0.003 |
| | | | | $F_Y < M_Y$ | 0.0007 |
| | | | | $F_N < M_N$ | 0.0001 |
| BALB/C × VY | | | N > Y | | <0.02 |
| | | N > Y | | | <0.04 |
| | | | | $F_Y = M_Y$ | >0.05 |
| | | | | $F_N > M_N$ | <0.02 |

[a] N = nonyellow.
[b] Y = yellow.
[c] F = female.
[d] M = male.

hair bulb melanocytes (17). However, in lean pseudoagouti $A^{vy}/a$ mice this pattern is disrupted in many individual hairs so that a wide variety of patterns is observed (16). Thus, the dysregulation of melanosome synthesis induced by the $A^{vy}$ mutation is still present, although to a much lesser degree than in the mottled yellow $A^{vy}/a$ mice. Similarly, the degree of susceptibility to lindane-induced enhancement of hepatocellular tumor formation was significantly less in pseudoagouti $A^{vy}/a$ (YS × VY) F-1 hybrid females than in their mottled yellow $A^{vy}/a$ siblings, but greater than in the congeneic black $a/a$ littermates (8).

Whereas prolonged lindane treatment induced hepatic glutathione *S*-transferase activity in a dose-dependent manner in CF-1 mice, it increased GST activity in B6C3F-1 females only at the highest tolerated dose (15). Lindane was reported to increase the prevalence of hepatocellular tumors in CF-1 mice but probably not in B6C3F-1 mice (15). This suggests that the degree to which GST activity was induced might be correlated with the enhancement of liver tumor formation by lindane. However, in contrast, our data indicate that, in female (YS × VY) F-1 hybrid mice, lindane at the highest tolerated dose decreased hepatic GST activity. Indeed, the lindane-related decrease in enzyme activity was similar in the black and pseudoagouti mice although they differ in susceptibility to lindane-related liver and lung tumor formation (8). Therefore, it appears that neither the level of hepatic GST activity per se nor the effect of lindane on this enzyme activity bear any direct relationship to lindane-related hepatic tumor formation.

The differential effects of the two $A^{vy}/a$ phenotypes on the CDNB-conjugating activity of hepatic GST illustrate the importance of phenotypic, as compared with genotypic, effects on enzyme activity. Table I indicates that in the obese mottled yellow $A^{vy}/a$ mice enzyme activity was distinctly less than that assayed in livers from lean pseudoagouti $A^{vy}/a$ and black $a/a$ females; whether this decrease resulted

from decreased enzyme synthesis, increased degradation, or other post-translational effects is not known. These data suggest that factors associated with obesity per se may inhibit the CDNB-conjugating activity. Alternatively, the inhibition may be a parallel effect, distinct from the obesity, of the phenotypic differentiation of the obese $A^{vy}/a$ phenotype. Characterization of the enzyme kinetics in these and other genetically obese mice, e.g., *ob/ob, db/db,* and their nonobese littermates would provide data for deciding between these alternatives.

Females of various species, including *Homo sapiens,* have higher GST activities than males (18, 19). This sex difference was also noted in the inbred AE and YS mouse strains and in the (BALB/c × VY) F-1 hybrid tested in this study. However, in the inbred VY and BALB/c strains, as in the outbred CF1 stock and B6C3F-1 hybrid (15), males had higher enzyme activity than the females.

Diametrically opposite sex/phenotype differences were found in the YS and VY strains (Table IV). In the YS strain black males had lower enzyme activity than black females; however, the obese yellow phenotype decreased the activity in females while apparently increasing it in males. In contrast, among VY strain mice, enzyme activity was *higher* in black males than in black females. It was about 50% lower in yellow VY females than in black *a/a* females but was *not* markedly lower in yellow than in black males. This was also observed among the yellow ($A^{vy}/a$) and nonyellow ($A/a$) (BALB/c × VY) F-1 hybrids.

Obviously there must be interplay among several physiological factors which affect enzyme activity to result in this complicated situation. The fact that sex–phenotype differences are dissimilar in the YS and VY strains suggests that the regulation of the synthesis, activity or degradation of regulatory factors which modulate the enzyme activity differs between the strains. The complexity of the regulation of the phenotypic expression of a single enzyme has been elegantly documented by Paigen and his co-workers (20). It is further illustrated by the induction by ethyl methanesulfonate of single mutations, each of which affects the activity of several apparently unrelated enzymes in the mouse (21).

The differential effects of the obese yellow phenotype on hepatic GST activity in the VY and YS strains are analogous to the differences in expression of the "obese" *ob/ob* and "diabetes" *db/db* genotypes in the inbred C57BL/6J and C57BL/KsJ strains (22). In all these cases it is the strain genome which specifies the altered response of the cells and tissues of the mouse to the primary dysregulation of cell metabolism induced by the mutant genotype. Thus, it is the interaction of the regulatory effect of the mutant gene(s) with the metabolic–physiological characteristics determined by the strain genome which results in the phenotypic expression of the mutant genotype at the organismic level. This concept is important not only for understanding the genetic control of metabolism but also for its implications for gene transfer between species by genetic engineering.

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1. Silvers WK. An experimental approach to action of the genes at the agouti locus in the mouse. II. Transplants of newborn *aa* ventral skin to $a^t a$, $A^w a$ and *aa* hosts. J Exp Zool **137**:181–188, 1958.
2. Meade CJ, Ashwell M, Sowter C. Is genetically transmitted obesity due to an adipose-tissue defect? Proc R Soc London B **205**:395–410, 1979.
3. Wolff GL. Stimulation of growth of transplantable tumors by genes which promote spontaneous tumor development. Cancer Res **30**:1731–1735, 1970.
4. Wolff GL, Medina D, Umholtz RL. Manifestation of hyperplastic alveolar nodules and mammary tumors in "viable yellow" and non-yellow mice. J Natl Cancer Inst **63**:781–785, 1979.
5. Wolff GL, Kodell RL, Cameron AM, Medina D. Accelerated appearance of chemically induced mammary carcinomas in obese yellow ($A^{vy}/A$) (BALB/c × VY)$F_1$ hybrid mice. J Toxicol Environ Health **10**:131–142, 1982.
6. Wolff GL. Controlled genetic variation in subchronic toxicity assays. NCTR Technical Report for Experiment No. 277: pp1–26, 1983.
7. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem **249**:7130–7139, 1974.
8. Wolff GL, Morrissey RL. Increased responsiveness of lean pseudoagouti $A^{vy}/a$ female mice to lindane en-

hancement of lung and liver tumorigenesis. Proc Amer Assoc Cancer Res **27,** 1986, in press.
9. Cranmer MF. Advances in animal care technology at the National Center for Toxicological Research. Lab Anim Sci **26:**355–373, 1976.
10. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys **82:**70–77, 1959.
11. Jollow DJ, Mitchell JR, Zampglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology **11:**151–169, 1974.
12. Bennett CA, Franklin NL. Statistical Analysis in Chemistry and the Chemical Industry. New York, Wiley, 1954.
13. Wolff GL, Suber RL. Influence of lindane and yellow ($A^{vy}/a$) phenotype on hepatic glutathione *S*-transferase activity in mice. Toxicologist **3:**77, 1983.
14. Wolff GL. Genetic modification of homeostatic regulation in the mouse. Amer Nat **105:**241–252, 1971.
15. Oesch F, Friedberg T, Herbst M, Paul W, Wilhelm N, Bentley P. Effects of lindane treatment on drug metabolizing enzymes and liver weight of CF1 mice in which it evoked hepatomas and in non-susceptible rodents. Chem Biol Interact **40:**1–14, 1982.
16. Galbraith DB, Wolff GL. Aberrant regulation of the agouti pigment pattern in the viable yellow ($A^{vy}/a$) mouse. J Hered **65:**137–140, 1974.
17. Sakurai T, Ochiai H, Takeuchi T. Ultrastructural change of melanosomes associated with agouti pattern formation in mouse hair. Dev Biol **47:**466–471, 1975.
18. Das M, Dixit R, Seth PK, Mukhtar H. Glutathione S-transferase activity in the brain: Species, sex, regional and age differences. J Neurochem **36:**1439–1442, 1981.
19. Strange RC, Johnson PH, Lawton A, Moult JA, Tector MJ, Tyminski RJ, Cotton W. Studies on the variability of glutathione S-transferase from human erythrocytes. Clin Chim Acta **120:**251–260, 1982.
20. Paigen K. Acid hydrolases as models of genetic control. Annu Rev Genet **13:**417–466, 1979.
21. Bishop JB, Feuers RJ. Development of a new biochemical mutation test in mice based upon measurement of enzyme activities. II. Test results with ethyl methanesulfonate (EMS). Mutat Res **95:**273–285, 1982.
22. Coleman DL. Obese and diabetes: Two mutant genes causing diabetes–obesity syndromes in the mouse. Diabetologia **14:**141–144, 1978.

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