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# Genetic analysis of liver catalase activity in two substrains of C57BL mice

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## 1. INTRODUCTION

Variation between inbred strains of mice in respect to liver catalase activity was reported by Greenstein & Andervont (1942). They noted that the catalase activity in the livers of mice of strain C57BL was only approximately half that of mice of other strains studied. It was, therefore, surprising when in a survey of a number of strains we noted that the activity in the livers of mice of our C57BL/He substrain was at a high level equal to that of mice of strains C3H/He, C3Hf/He, YBR/He, and BALB/cDe (Rechcigl & Heston, 1963). We then investigated substrain C57BL/6 and found that the results for this substrain confirmed Greenstein and Andervont's earlier observation in that the level of liver catalase activity was approximately half that in C57BL/He and in the other inbred strains.

These observations prompted a more thorough survey of the liver and kidney catalase activity of various C57 strains and substrains and of strain C58, all of which belong to the family of strains derived from the original Lathrop stock (see Heston, 1949). We found that these strains and substrains did not vary in respect to kidney catalase activity. The strains and substrains low in liver catalase activity were C58, C57BR/cd, C57L, C57BL/10, C57BL/6, and C57BL/Sp, while substrains C57BL/An and C57BL/He had a high liver catalase activity. Because C57BL/He had been derived from C57BL/An which had come from C57BL/Sp it was postulated, under the assumption of a major gene affecting liver catalase activity, that a mutation had occurred in the progenitors of the C57BL/An substrain.

A thorough genetic analysis of the variation between substrains C57BL/He and C57BL/6 was, therefore, undertaken and the results are presented here. The difference in liver catalase activity was shown to be a single gene difference, surprisingly with low level of activity dominant to high. The gene did not affect the level of kidney catalase activity.

## 2. MATERIALS AND METHODS

The relationship of the various strains and substrains derived from the Lathrop stock has been outlined previously (Rechcigl & Heston, 1963).

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Our substrain C57BL/He (B/He), one of the parent substrains of the present study, was derived from Dr H. B. Andervont's substrain C57BL/An in 1945 at the thirtieth generation of brother × sister inbreeding of the substrain in his laboratory. Inbreeding of the C57BL/He was continued in this laboratory without a break in designation of generations and those animals used in the present study were of the $F_{62}$ and $F_{63}$ generations. Andervont's substrain C57BL/An was originated from C57BL mice obtained from W. S. Murray in 1939 of a line now continued by Simpson and designated as C57BL/Sp.

Animals of the substrain C57BL/6 (B/6), the other parent substrain of the present study, were derived from a breeding litter obtained in February, 1962, from a colony of C57BL/6 that one of us (H. Hoffman), who was then in the Laboratory Aids Branch of the National Institutes of Health, was maintaining at that time. This litter was of the $F_{66}$ generation of brother × sister inbreeding and the animals used in the present study were of this litter and the $F_{67-69}$ generations. Common ancestry of C57BL/He and C57BL/6 would have had to be sometime prior to 1939.

Reciprocal crosses were made between these parental substrains to produce the reciprocal $F_1$'s (Figs. 1 and 2). These $F_1$'s were in turn mated to produce reciprocal $F_2$'s and were also backcrossed to the parental substrains to produce reciprocal backcrosses. In addition, progeny tests were made of C57BL/He—first backcross segregant males by mating them with C57BL/He females.

Approximately fifty males and fifty females of the two parental substrains, the reciprocal $F_1$, and first backcross groups were produced for measuring catalase activity. Somewhat greater numbers were produced in the $F_2$ groups. The animals were weaned and individually marked at 4 weeks of age, segregated as to sex and set aside in plastic cages with eight mice to the cage. Throughout the study the animals were fed NCI pellets, the formula of which has been published (Heston *et al.*, 1960) and given tap water *ad libitum*.

Determination of liver and kidney catalase was made on all mice at 6 weeks of age except for those used for breeders which were analyzed at older ages when no longer needed for breeding. The mice were killed by cervical dislocation and the livers and kidneys were removed, weighed, and homogenized for 2 min. in a Waring blender with 99 vol. of ice-cold distilled water. Catalase was assayed spectrophotometrically by a modification (Rechcigl *et al.*, 1962) of the method of Beers & Sizer (1952) on a continuously recording spectrophotometer (Cary Model 11 MS Recording Spectrophotometer from the Applied Physics Corporation, Pasadena, California) with a log-absorbance attachment (Modified Brown Recorder, Applied Physics Corporation, Pasadena, California). To carry out the assays, 50 $\mu$l. samples of the homogenates were added to 2 cuvettes containing 2·9 ml. of 0·02 M phosphate buffer at pH 6·8 and 22°C. The pen of the spectrophotometer was then set at the baseline, and 30 $\mu$l. of 1·0 M hydrogen peroxide were rapidly added to the experimental cuvette with the adder-mixer described by Boyer & Segal (1954). Recording was started immediately, and the decrease in optical density at 230 $\mu$ was traced directly onto semilogarithmic paper. From the slope of the line obtained,

the first order constant, $k_0$, for a given determination, was calculated. One unit of catalase is sufficient enzyme to split 63·2% of a given concentration of $H_2O_2$ in 1 sec. and is equivalent to 5·85 $\mu g$ of purified rat liver catalase.

### 3. ANALYSIS OF RESULTS

In Fig. 1 it can be observed that there was no variation between the two parental substrains and the hybrid groups in level of kidney catalase activity. Throughout the groups, however, there was a very striking uniform sex difference as had been noted previously, the level for the females being only about half that for the males. A sex difference in level of liver catalase is also apparent in all groups (Fig. 2), although not nearly as great as the sex difference in kidney catalase activity.

The difference between the two substrains in respect to liver catalase activity is clearly shown in Fig. 2 and Table 1. The means for the male and female groups of substrain C57BL/6 were approximately half the means for the male and female groups of substrain C57BL/He and there was no overlap between the two substrains.

Dominance of low liver catalase activity over high was evident in that the $F_1$ groups were in the range of the C57BL/6 groups and did not overlap with the C57BL/He groups. There was no evidence of any maternal influence for the reciprocal $F_1$'s were alike. When the reciprocal $F_1$ groups were combined, the mean value for the males was slightly but statistically significantly higher ($P < 0{\cdot}01$) than that for the C57BL/6. This may have been chance variation or it may indicate slightly incomplete dominance of low level activity. If there is incomplete dominance, however, it did not appear in the females, for the mean of the $F_1$ females was almost the same as that of the C57BL/6 females.

Evidence of segregation was apparent in that there was much greater variation in the $F_2$ groups than in the $F_1$ or parental groups. Furthermore, the distribution in the $F_2$ groups was distinctly bimodal, indicating segregation of a single pair of genes. The distribution within the groups resulting from backcrossing the $F_1$'s reciprocally to the C57BL/6 was unimodal and approximately within the range of the C57BL/6 and $F_1$ groups, confirming the dominance of low level activity. The variation within the groups resulting from backcrossing the $F_1$'s reciprocally to the C57BL/He parental substrain like that in the $F_2$ groups was greater than that in either parental substrain or the $F_1$'s and the distribution within each of these backcross groups was bimodal, also suggesting that but a single pair of genes was involved.

The cutoff values for high and low level of liver catalase activity for the $F_2$ generation and the recessive-parent backcross were determined by the posterior odds method for univariate normal classifications of Geisser (1964). The method takes into account the observed catalase distributions of the defined genotypic groups—the inbred C57BL/6 and C57BL/He substrains and their $F_1$ hybrid—and the *a priori* probabilities of $F_2$ or backcross segregants based on an hypothesis of single gene control. The simplifying assumption was made that the distributions of the two inbred strains and their $F_1$ hybrid represent the range of activities of the gene combinations segregating in the $F_2$ and backcross groups.

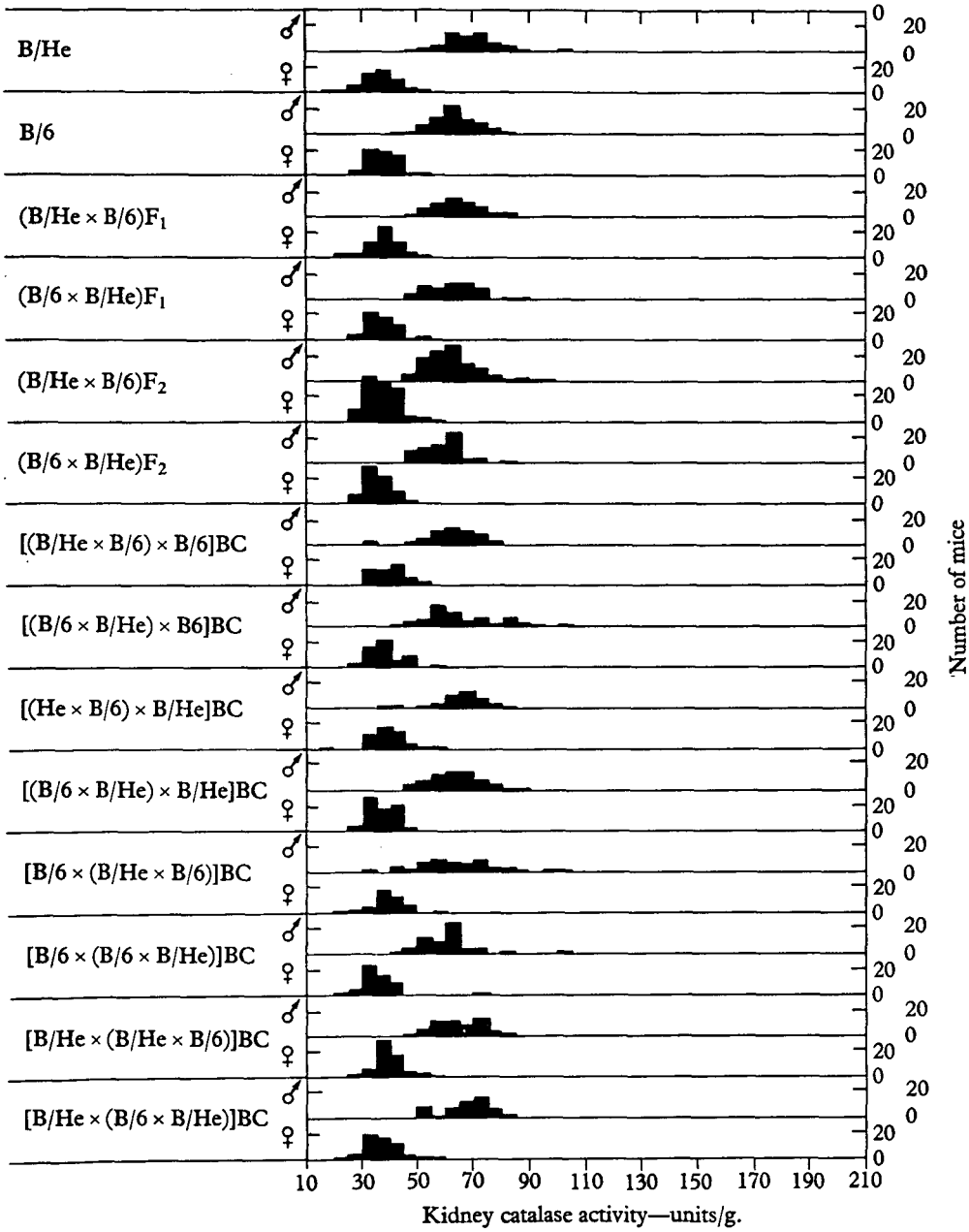


Fig. 1. Distribution of mice of strains C57BL/He (B/He) and C57BL/6 (B/6) and their hybrids in respect to kidney catalase activity.

The analysis was carried out in two steps; first, for each catalase activity value the likelihood (predictive density) that an activity value belongs to each genotypic group separately was computed. Then for each activity value a likelihood ratio

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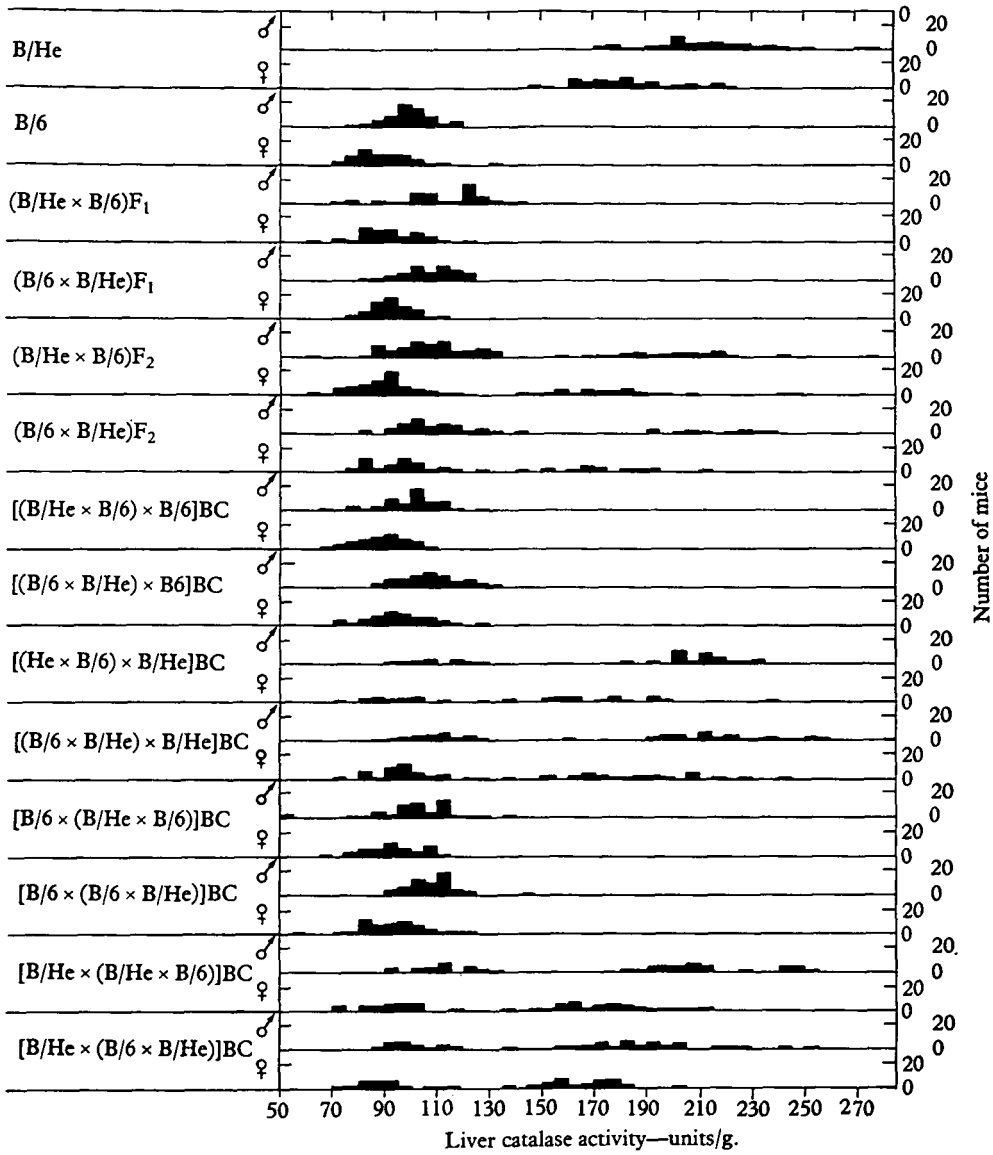


Fig. 2. Distribution of mice of strains C57BL/He (B/He) and C57BL/6 (B/6) and their hybrids in respect to liver catalase activity.

of the predictive densities was computed, and the cutoff value for high and low catalase activity was set where the likelihood ratios of the activity values were maximum for each genotypic group. The predictive density was then combined with the *a priori* probability of a segregating class in order to obtain the probability (posterior probability) that the activity value belonged to a specific genotypic group and segregating class.

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Table 1. *Liver catalase activity in substrains C57BL/He, C57BL/6, and their hybrids*

| Group | Sex | Number of mice | Level of catalase activity | Liver catalase units/gram Mean ± S.E. | Coefficient of variation (per cent) |
|---|---|---|---|---|---|
| C57BL/He | ♂ | 58 | High | 213·0 ± 2·8 | 10·0 |
| | ♀ | 51 | High | 180·9 ± 2·6 | 10·3 |
| C57BL/6 | ♂ | 62 | Low | 99·2 ± 1·1 | 8·7 |
| | ♀ | 54 | Low | 89·4 ± 1·5 | 12·6 |
| $F_1$ | ♂ | 103 | Low | 109·7 ± 1·3 | 11·9 |
| | ♀ | 104 | Low | 91·8 ± 0·9 | 10·1 |
| $F_2$ | ♂ | 125 | Low | 107·1 ± 1·2 | 12·6 |
| | | 36 | High | 204·8 ± 3·7 | 10·9 |
| | ♀ | 115 | Low | 91·6 ± 1·1 | 13·2 |
| | | 48 | High | 174·6 ± 3·2 | 12·8 |
| $F_1 \times$ B/6 | ♂ | 203 | Low | 104·4 ± 0·8 | 11 0 |
| | ♀ | 190 | Low | 92·4 ± 0·8 | 11·7 |
| $F_1 \times$ B/He | ♂ | 84 | Low | 108·0 ± 1·2 | 10·4 |
| | | 118 | High | 208·2 ± 2·1 | 11·0 |
| | ♀ | 102 | Low | 93·0 ± 1·0 | 11·4 |
| | | 118 | High | 172·0 ± 2·0 | 12·4 |
| $(F_1 \times$ B/He$) \times$ B/He | ♂ | 59 | Low | 104·9 ± 1·2 | 9·0 |
| | | 107 | High | 202·0 ± 2·4 | 12·1 |
| | ♀ | 54 | Low | 93·6 ± 1·5 | 11·9 |
| | | 97 | High | 174·5 ± 1·6 | 8·9 |

Predictive densities for catalase activity values from 70 to 230 were computed by formula 2.3 of Geisser:

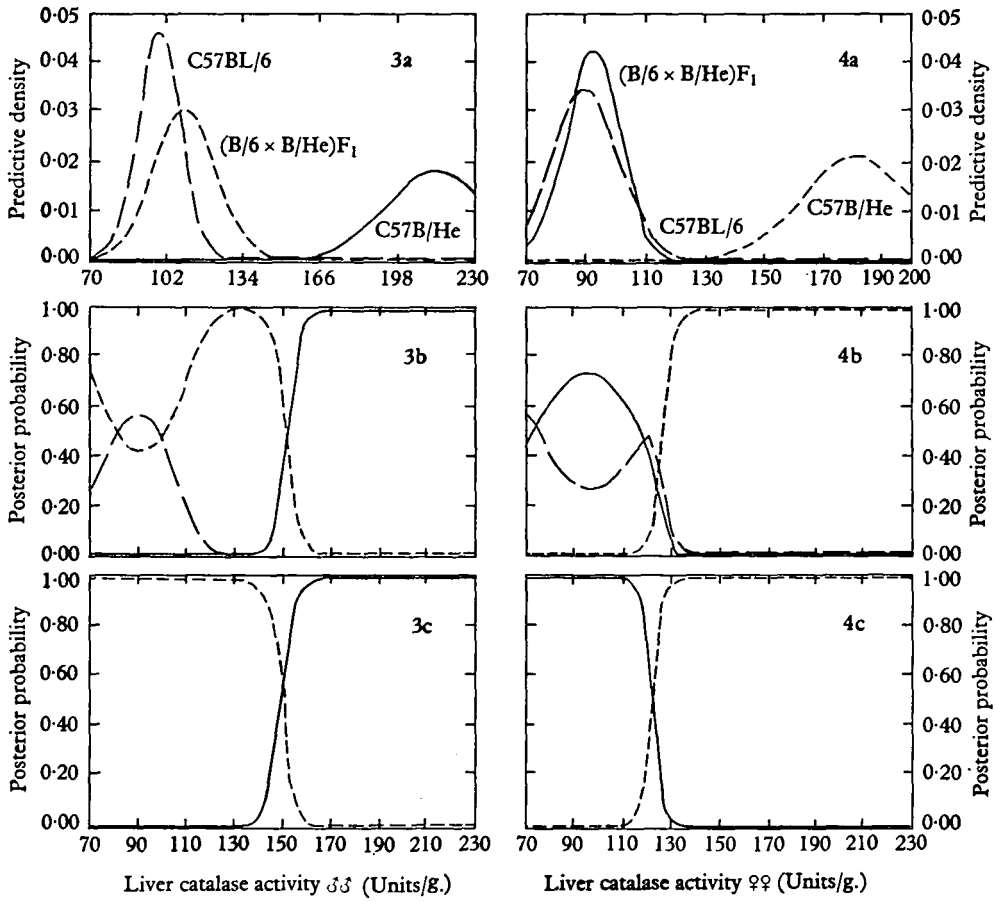
$$f(z \mid \bar{x}_i, s_i, G_i) = \left(\frac{N_i}{(N_i^2-1)\,\Pi}\right)^{1/2} \frac{\Gamma\left(\frac{N_i}{2}\right)}{\Gamma\left(\frac{N_i-1}{2}\right) s_i} \left[1+\frac{N_i(\bar{x}_i-z)^2}{(N_i^2-1)\,s_i^2}\right]^{-N_i/2}$$

where $z$ is the catalase activity value, $N_i$, $\bar{x}_i$, and $s_i$ are the sample size, mean, and standard deviation, respectively, of the genotypic group $G_i$. Distributions of the predictive densities for the three genotypic groups are shown in Figs. 3*a* and 4*a* for males and females, respectively. Posterior probabilities were computed by formula 2.5 of Geisser:

$$P(G_i \mid z, q) = \frac{q_i f(z \mid \bar{x}_i, s_i, G_i)}{\sum_j q_j f(z \mid \bar{x}_j, s_j, G_j)}$$

where $q_i$ is the *a priori* probability of a segregation class and $\sum q_i = 1$.

The male cutoff value for high and low catalase activity for $F_2$ and recessive-parent backcross segregation was set at 150·5. Above this catalase activity value the likelihood ratios of the high activity C57BL/He strain are greater than 0·5




Figs. 3 and 4. (*a*) Distributions of predictive densities for liver catalase activity for the C57BL/6, C57BL/He, and $F_1$ hybrid; (*b*) posterior probabilities for $F_2$—with *a priori* probabilities 1/4 for C57BL/6, 1/4 for C57BL/He, and 1/2 for the $F_1$ hybrid; (*c*) posterior probabilities for C57BL/He-backcross—with *a priori* probabilities 0 for C57BL/6, 1/2 for C57BL/He, and 1/2 for the $F_1$ hybrid.

Fig. 3: males. Fig. 4: females.

and are less than 0·5 below this value. Below 150·5 the sum of the values of the likelihood ratios of the low activity groups, the C57BL/6 and $(B/6 \times B/He)F_1$, is greater than 0·5 and less than 0·5 above the cutoff value. It can be noted from a comparison of Figs. 3*a* and 3*b* that the distributions of posterior probabilities of the low and high catalase groups are in good agreement with the 150·5 cutoff value. The *a priori* probabilities for the curves in Fig. 3*b* were 1/4:1/4:1/2 for the C57BL/6, C57BL/He, and $F_1$ groups, respectively. Any errors of classification as assessed by the posterior probabilities will be close to the 150·5 cutoff value. For example, in the $F_2$ generation the probabilities that catalase values 150, 151, and 152 belong to the high catalase group are 0·32, 0·40, and 0.48, respectively (Fig. 3*b*). In the recessive-parent backcross generation, the probabilities that catalase values 150 and 151 belong to the high activity group are 0·48 and 0·57,

respectively (Fig. 3*c*). The *a priori* probabilities for the curves in Fig. 3*c* were 0:1/2:1/2 for the C57BL/6, C57BL/He, and $F_1$ groups.

The female cutoff value for high and low catalase activity of 125·5 was determined in the identical way as the males. It can be noted from Figs. 4 *a*, 4*b*, and 4*c* that the distributions of the posterior probabilities of the $F_2$ and recessive-parent backcross generations are in agreement with the 125·5 cutoff value.

The distribution of the segregating $F_2$ and C57BL/He-backcross groups into high and low when these cutoff values were applied is shown in Table 2. The distribution of low and high in the $F_2$ males and females did not deviate significantly from the 3:1 ratio expected if the level of liver catalase activity is controlled by a single gene pair with low dominant to high. When the data for the sexes were combined the distribution was 240 low to 84 high, which is very close to the expected 243–81.

In comparing the distribution of the C57BL/He backcross groups with the 1:1 ratio expected with single gene inheritance and with low dominant to high there appeared to be an excess of high males. The probability that the observed 84 low to 118 high males represented a chance variation from the expected 101 to 101 was between 0·01 and 0·05. The distribution of the females, however, did not differ significantly from the 1:1 ratio. When the data for the males and females were combined the probability that the observed distribution of 186 low to 236 high was a chance deviation from the expected 211 to 211 was 0·01 to 0·05. It would appear, therefore, that the deviation in the male group from the 1:1 ratio was a chance deviation or was due to some factors other than genetic ones.

Table 2. *Comparison of observed segregation in $F_2$ and $F_1 \times B/He$ backcross with the expected single gene segregation*

| | | Observed | | Expected | | |
|---|---|---|---|---|---|---|
| Hybrid | Sex | Low | High | Low | High | *P* |
| $F_2$ | ♂ | 125 | 36 | 121 | 40 | 0·3 –0·5 |
| | ♀ | 115 | 48 | 122 | 41 | 0·1 –0·2 |
| | ♂ & ♀ | 240 | 84 | 243 | 81 | 0·5 –0·7 |
| $F_1 \times$ B/He | ♂ | 84 | 118 | 101 | 101 | 0·01–0·05 |
| | ♀ | 102 | 118 | 110 | 110 | 0·2 –0·5 |
| | ♂ & ♀ | 186 | 236 | 211 | 211 | 0·01–0·05 |

Confirming single factor inheritance with low dominant to high are the results of the progeny tests of the seventeen C57BL/He-first backcross segregant males that are presented in Table 3. With this pattern of inheritance the offspring of these first backcross males that were in turn backcrossed to C57BL/He females should identify these males as belonging to two groups, homozygous high males that should have high offspring only and heterozygous low males that should have

Table 3. *Progeny test of C51BL/He-backcross segregants*

| Backcross ♂♂ | | Offspring | | | |
|---|---|---|---|---|---|
| | | High catalase | | Low catalase | |
| Male's number | Liver catalase (units/g.) | ♂ | ♀ | ♂ | ♀ |
| 94089 | 118 | 5 | 4 | 3 | 2 |
| 93725 | 105 | 5 | 3 | 5 | 4 |
| 93885 | 116 | 3 | 1 | 3 | 4 |
| 93727 | 110 | 6 | 4 | 5 | 3 |
| 93726 | 120 | 4 | 7 | 3 | 5 |
| 94088 | 110 | 2 | 3 | 5 | 5 |
| 94153 | 107 | 1 | 4 | 1 | 8 |
| 96022 | 104 | 4 | 3 | 10 | 2 |
| 96117 | 94 | 9 | 2 | 5 | 5 |
| 96118 | 92 | 4 | 5 | 6 | 6 |
| 96119 | 100 | 2 | 9 | 7 | 5 |
| 96124 | 116 | 5 | 4 | 6 | 5 |
| 94154 | 204 | 8 | 9 | 0 | 0 |
| 93886 | 202 | 11 | 10 | 0 | 0 |
| 96116 | 184 | 15 | 10 | 0 | 0 |
| 96120 | 174 | 10 | 9 | 0 | 0 |
| 96123 | 162 | 13 | 10 | 0 | 0 |

offspring half of which were low and half of which were high. From Table 3 one notes that they were thus divided. Five males had nothing but offspring with high liver catalase activity, whereas approximately half the offspring of each of the remaining twelve were high and half low. The total number of offspring of these twelve males was 230 and of these 117 were high and 113 low, a very close approximation of the expected 1:1 ratio.

Confirmation of the genotypes of the seventeen males as determined by the progeny tests was later obtained when they were killed and their livers were analyzed for catalase activity. The five whose breeding tests had indicated that they were homozygous high all showed a high level of liver catalase activity as noted in Table 3, whereas the twelve indicated as heterozygous low all had low liver catalase activity.

## 4. DISCUSSION

The data presented show and confirm that the level of liver catalase activity in the mouse is controlled by a single pair of genes with low dominant to high. We are suggesting *Ce* for the gene symbol. Thus, C57BL/6 would be *CeCe*, C57BL/He *cece*, and the $F_1$ hybrid *Cece*.

In considering the possible mode of action of this gene one notes several points that suggest that the gene does not act directly in the synthesis of catalase. First, the presence of the gene reduces catalase activity only to one-half the normal

amount rather than eliminating activity; the homozygous and heterozygous low both being expressed at this level. Second, low is dominant to high in contrast with genetic control over direct synthesis of enzymes. Third, the effect of the gene is apparently limited to the liver; in this study the level of activity in the kidney certainly was not affected. Thus, the gene must be providing a regulator of the amount of catalase activity in the liver. This is in sharp contrast with acatalasemia in the Japanese where the gene is apparently involved directly in the synthesis of catalase, for homozygous recessive individuals show no evidence of catalase (Takahara *et al.*, 1960; Hamilton *et al.*, 1963).*

Inasmuch as most strains have a high level of liver catalase activity and the wild mice we have tested likewise had a high level of activity, the recessive high should be considered the wild-type. The original mutation then would have been a dominant mutation to the low level that appeared in the original Lathrop stock from which the C57 and C58 lines arose. Since all of these lines were low it is probable that the original females number 57 and 58 and their littermate male number 52 that were mated in 1921 were homozygous for the mutation. If not, the mutation would have been fixed in the homozygous state through the subsequent years of brother × sister inbreeding. A reverse mutation back to the recessive wild-type must then have occurred in the immediate progenitors of Andervont's subline of strain C57BL, and by selection the homozygous recessive was established in Andervont's C57BL/An and in the C57BL/He substrain derived from it.

Linkage studies to locate the gene would be the next appropriate step. With biochemical genetics in the mouse developing so rapidly this gene should provide an excellent biochemical marker.

## SUMMARY

The level of liver catalase activity in substrain C57BL/6 mice is only approximately half that in substrain C57BL/He. That this difference was due to a single major gene with low level of activity dominant to high was indicated by analysis of the $F_1$, $F_2$, and backcross hybrids and confirmed by progeny tests of first backcross male segregants. The gene symbol *Ce* is suggested.

The absence of any difference between reciprocal hybrids indicated no extrachromosomal maternal influence.

There was no evidence that this gene controlling liver catalase had any influence on the level of kidney catalase activity for this did not vary between the two parent substrains or the hybrid groups.

Males consistently had higher liver and kidney catalase activity than females. The difference was more pronounced in respect to kidney catalase, males having about twice the activity found in females.

* Subsequent to the submission of this paper preliminary results by R. N. Feinstein, J. E. Seaholm, J. B. Howard, and W. L. Russell have appeared which suggest that acatalasemia similar to that in man may appear also in the mouse. (See *Proc. natn. Acad. Sci. U.S.A.* **54**, 661–662, 1964.)

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