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A major autosomal gene effect on activity of glucose 6-phosphate dehydrogenase segregating between recombinant inbred lines of mice

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SUMMARY

G6PD measurements (expressed per g haemoglobin) in both males and females of the parental lines C57BL/6J and C57L/J and four recombinant inbred (RI) lines between them showed segregation of G6PD levels as parental classes (one line showed the higher C57BL/6J activity, three lines were indistinguishable from C57L/J). Haemoglobin also varied significantly among the parental and RI lines but this variation did not explain the variation in G6PD. NADP levels (expressed per g wet wt of erythrocytes) correlated with G6PD levels but one RI line was intermediate in males while two RI lines were lower than either parental line in females. Thus, the major autosomal locus affecting G6PD levels which segregates between these two inbred lines probably does not act directly on NADP levels. The variation in the G6PD levels in the four RI lines co-segregated with the brown coat color locus but the association is not significant because of the small number of RI lines available.

1. INTRODUCTION

Glucose 6-phosphate dehydrogenase (G6PD, D-glucose 6-phosphate: NADP oxidoreductase, E.C. 1.1.1.49) is specified by an *X*-linked structural gene in mice (Chapman & Shows, 1976) as in man. Erythrocytic levels of G6PD vary markedly among inbred strains of mice. Hutton (1971) has shown that these genetic differences can only be explained by 2 or more autosomal loci. Erickson (1974) has previously shown that the strains with low G6PD activity have low erythrocytic levels of nicotinamide-adenine dinucleotide phosphate (NADP) but strain differences in NADP levels did not explain the differences in erythrocytic G6PD activity between the high and intermediate strains. An effect of sex and other variables precluded a strong demonstration of unitary genetic control in crosses between intermediate and low strains. The availability of a small number of recombinant inbred (RI) lines from a cross of an intermediate (C57BL/6J) by a low (C57L/J) strain allowed an analysis of the genetic control of erythrocytic G6PD and NADP differences between these strains. A major autosomal gene regulating erythrocytic G6PD which happened to co-segregate with the brown

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locus was identified. Erythrocytic NADP levels tended to parallel the G6PD levels.

2. MATERIALS AND METHODS

(i) *Animals*

Inbred mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Breeding stock for the LXB RI lines were the kind gift of Benjamin A. Taylor, The Jackson Laboratory, Bar Harbor, Maine. Shavings (1/3 cedar) were used as bedding and the mice were kept in a room with controlled lighting (14 h light, 10 h dark). Animals were used when they were between 10 and 40 weeks of age. All three assays were done on each mouse.

(ii) *Erythrocytes*

All biochemical measurements were made with blood obtained by intra-cardiac puncture of ether-anesthetized mice. Haemoglobin and G6PD levels were assayed with whole heparinized blood. Blood for the NADP assays was pelleted in a 10X volume of 0·9 % NaCl–0·1 % glucose solution at 700Xg for 10 min, at 0–4 °C in preweighed plastic centrifuge tubes. The buffy coat and the supernatant were removed from the pellet and the remaining red blood cells were treated with 5 % TCA for NADP extraction.

(iii) *Determinations*

All biochemicals were the best grade available from Sigma Chemical Co., St Louis, Mo. NADP was assayed by a micromodification (Erickson *et al.* 1972) of the standard method of Ciotti & Kaplan (1957). G6PD was assayed by a micromethod (Epstein, Wegienka & Smith, 1969). Haemoglobin concentrations were determined by the cyanomethemoglobin method (Oser, 1965) using crystalline bovine haemoglobin (Sigma, type 1) as a standard.

(iv) *Statistical analyses*

A one-way analysis of variance was performed with the F test of the equality of different means. Schaffe's multiple comparison procedure was used to contrast specific linear combinations of strain means. Two-tailed *t*-tests of the significance of correlation coefficients were also performed.

3. RESULTS

(i) *Erythrocytic G6PD levels*

Erythrocytic G6PD levels were measured in the four recombinant inbred lines available from the cross of the C57L/J and C57BL/6J strains and in these two parental strains. In males, the LXB-3 RI line was not different than the higher activity level parental strain, C57BL/6J, while the other three RI lines (LXB-1, LXB-2, LXB-4) showed G6PD levels similar to those of the low activity, C57L/J strain (Table 1). There is a statistically significant difference between the extremes

of this latter group but all four strains are highly different from the first two strains. The pattern of G6PD activity among the female lines closely parallels that of the male lines (Table 2). LXB-3 is close in value to C57BL/6J, (although

Table 1. *Male mouse erythrocyte G6PD levels*

Strain	Number	μmole/g. hgb/min		
C57BL/6J	11	25·47 ± 0·97*	N.S.	$P \ll 0{\cdot}01$
LXB-3	9	26·18 ± 1·67		
LXB-1	6	14·86 ± 0·37	$P < 0{\cdot}03$	
LXB-2	6	15·85 ± 1·01		
LXB-4	9	11·92 ± 0·69		
C57L/J	6	12·48 ± 1·48		

* Mean ± S.E.

Table 2. *Female mouse erythrocyte G6PD levels*

Strain	Number	μmole/g. hgb/min		
C57BL/6J	11	23·16 ± 0·99*	$P < 0{\cdot}03$	$P \ll 0{\cdot}001$
LXB-3	8	26·89 ± 1·99		
LXB-1	5	13·07 ± 0·81	N.S.	
LXB-2	6	13·00 ± 0·40		
LXB-4	7	11·95 ± 0·64		
C57L/J	5	11·20 ± 1·39		

* Mean ± S.E.

the difference reaches significance) while the other RI lines are not distinguishable from C57L/J. These two groupings (the high strain and one RI line vs. the low strain and three RI lines) are very significantly different from one another. These erythrocyte G6PD levels correlate with the brown coat colour locus: LXB-3 is +/+ as is C57BL/6J; LXB-1, -2, and -4 are b/b as is C57L/J.

(ii) *Variation in haemoglobin levels*

Since the G6PD levels are expressed per amount of haemoglobin, it seemed important to investigate whether there was genetic heterogeneity in haemoglobin levels. A fair amount of variation was found in haemoglobin levels among males with the two strains which showed high levels of G6PD (expressed per g hgb) having low levels of haemoglobin while two strains with low levels of G6PD (LXB-4 and C57L/J) showing high levels of haemoglobin (Table 3). However, two other strains with indistinguishably low levels of G6PD had intermediate levels of haemoglobin. Haemoglobin levels of female mice of the six strains also showed genetic heterogeneity which would only partially explain G6PD levels (Table 4). In female mice, haemoglobin levels of the two high G6PD strains and two of the low G6PD strains were not distinguishable. However, the other two low G6PD strains had higher levels of haemoglobin than the other four strains. Although the haemoglobin levels of the high G6PD strains LXB-3 and C57BL/6J were not distinguishable from the haemoglobin levels of the LXB-1 and LXB-2

Table 3. *Male mouse haemoglobin levels*

Strain	Number	g. hgb/ml		
C57BL/6J	11	0·329 ± 0·009*	N.S.	$P < 0{\cdot}01$
LXB-3	9	0·331 ± 0·009		
LXB-1	6	0·358 ± 0·014		
LXB-2	6	0·344 ± 0·016		
LXB-4	9	0·371 ± 0·009	N.S.	
C57L/J	6	0·390 ± 0·021		

* Mean ± S.E.

Table 4. *Female mouse haemoglobin levels*

Strain	Number	g. hgb/ml		
C57BL/6J	11	0·321 ± 0·012*	N.S.	$P < 0{\cdot}01$
LXB-3	8	0·316 ± 0·005		
LXB-1	5	0·331 ± 0·012		
LXB-2	6	0·324 ± 0·011		
LXB-4	7	0·357 ± 0·014		
C57L/J	5	0·385 ± 0·014		

* Mean ± S.E.

low G6PD strains, there was no overlap in the ranges of G6PD for the two groups.

(iii) *Erythrocytic NADP levels*

The values for NADP levels tended to parallel those found for G6PD. Males of three of the RI lines (LXB-1, LXB-2, and LXB-4) are not demonstrably different from the low parental strain (C57L/J) while the fourth RI line (LXB-3) has levels midway between the low and the high parental line (C57BL/6J) (Table 5). For females, one of the RI lines (LXB-3) was indistinguishable from C57BL/6J while one (LXB-4) was indistinguishable from C57L/J (Table 6). The remaining two strains had levels much lower than either parental strain – levels comparable to low male strains. The values for males of the parental strains (Table 5) are now somewhat higher than previously reported (Erickson, 1974). The values for females of the parental strains are now a great deal higher than previously reported (Erickson, 1974); more so for C57L/J (nearly doubled) than for C57BL/6J.

(iv) *Correlation of erythrocytic G6PD and NADP*

Separate calculations in each sex of correlation coefficients for NADP and G6PD for the individuals in the 6 strains demonstrate a stronger correlation of NADP and G6PD in males than in females. The correlation coefficient (r) was 0·644 in males (R @ 0·01 = 0·372, thus, the hypothesis that r = 0 is rejected) but only 0·548 (R @ 0·01 = 0·3932, thus, the hypothesis that r = 0 is rejected) in females.

Table 5. *Male mouse erythrocyte NADP levels*

Strain	Number	nmole/g. red blood cells		
C57BL/6J	11	25·57 ± 1·50*	$P < 0{\cdot}02$	$P < 0{\cdot}04$
LXB-3	9	20·81 ± 1·86		
LXB-1	6	12·367 ± 0·91	N.S.	
LXB-2	6	12·22 ± 1·11		
LXB-4	9	15·68 ± 1·68		
C57L/J	6	15·72 ± 1·57		

* Mean ± S.E.

Table 6. *Female mouse erythrocyte NADP levels*

Strain	Number	nmole/g. red blood cells			
C57BL/6J	11	28·14 ± 1·76*	N.S.	$P < 0{\cdot}01$	
LXB-3	8	30·95 ± 2·09			
LXB-1	5	15·17 ± 2·13	N.S.		$P < 0{\cdot}02$
LXB-2	6	11·52 ± 1·24			
LXB-4	7	22·59 ± 1·80	N.S.		
C57L/J	5	24·84 ± 2·36			

* Mean ± S.E.

4. DISCUSSION

Recombinant inbred lines provide a powerful tool for studying the genetic control of biochemical variables for which there is significant variation in the assay itself (Bailey, 1971). The availability of a number of genetically identical individuals, each RI line representing a F2 recombination of alleles, allows repetition of assays until assay variability can be largely eliminated as a compounding variable (Swank & Bailey, 1973). We have used the four RI lines available between the C57BL/6J strain of mice, which has an intermediate level of glucose 6-phosphate dehydrogenase and an intermediate level of nicotinamide-adenine dinucleotide phosphate, and C57L/J, which has low levels of these two variables (Erickson, 1974). We found that the G6PD levels in these four recombinant inbred lines segregated as parental levels of activity. This result is most compatible with alleles at a single locus determining this variation in G6PD activity. However, the number of RI lines studied is too small to be conclusive. A fifth RI line was originally maintained for this cross but it has a low viability and is not available to us.

The G6PD levels were determined per g haemoglobin. The previously described strain variation in haemoglobin levels between C57BL/6J and C57L/J (Russell, Neufeld & Higgins, 1951) was confirmed. The difference did not segregate as alleles of a single gene in the RI lines and the variation in haemoglobin levels did not explain the variation in G6PD levels.

The autosomal gene regulating erythrocytic G6PD shows the same strain distribution pattern as brown (*b*). The small number of strains involved prevents

any conclusion in regards to linkage. If the G6PD regulating locus is linked to *b* on chromosome 4, it seems likely that a new locus is being detected rather than a new effect of an already known locus. Possibly related loci on chromosome 4 (anaemia, *an*, 8 cMo proximal to *b*; phosphoglucose mutase 2, *pgm*-2, 9 cMo distal to *b*) are unlikely to have been fixed with *b* during the creation of these RI lines.

Of the many factors which affect the stability of G6PD, the most important seem to be those acting on the NADP:NADPH ratio (reviewed in Erickson, 1974). We have previously shown by heat inactivation tests of G6PD that the NAPD differences found among inbred strains of mice could explain the previously demonstrated lability of G6PD in the erythrocytes of a low G6PD activity strain C57L/J (Erickson, 1974). Erythrocytic G6PD activity and NADP levels in individual backcross mice correlated significantly (Erickson, 1974). In the present work, NADP levels tended to correlate with G6PD levels, but NADP levels did not completely segregate into parental classes in the four RI lines. Other loci must also be involved in determining erythrocytic NADP levels, e.g. the gene(s) which determines the sexual dimorphism in erythrocytic NADP levels observed in the C57BL/6J strain. The NADP:NADPH ratio may be the important factor under genetic control which determines the variation in G6PD levels, i.e. it is possible that the NADP redox potential in LXB-3 males is identical to that in C57BL/6J males but that the total pool of oxidized and reduced NADP is lower in LXB-3 males while the redox potential of NADP would be lower in the three other RI lines and C57L/J. On this hypothesis the levels of oxidized and reduced levels of NADP in females of the LXB-1 and LXB-2 lines would be expected to be lower than in the LXB-4 and C57L/J lines while the redox potential would remain the same.

Another factor known to effect levels of enzymes in erythrocytes is red blood cell age – older cells having lower levels of enzyme. Although red blood cell survival studies have not been performed in these two strains, the identical reticulocyte counts in C57BL/6J and C57L/J (Russell *et al.* 1951) suggest that there are not large differences.

We conclude that genetic variation in the activity of an enzyme does not necessarily reflect allelic mutation at the structural locus. Variations in corticosterone reduction between C57BL/N and DBA/2 mice partially reflect different concentrations of NADPH (Shire, Kessler & Clayton, 1972) and these differences in co-factor concentration may play a role in differences in microsomal electron transport in the two strains (Blumer & Mieyal, 1978).

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