IDENTIFICATION OF A MAJOR LOCUS CONTRIBUTING TO
ERYTHROCYTE 2,3-DIPHOSPHOGLYCERATE VARIABILITY IN
HOODED (LONG-EVANS) RATS¹

N. A. NOBLE² AND G. J. BREWER

Department of Human Genetics, University of Michigan Medical School,
Ann Arbor, Michigan 48109

Manuscript received September 9, 1976
Revised copy received January 5, 1977

ABSTRACT

The erythrocyte glycolytic intermediate 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) play an important role in oxygen transport and delivery by binding to hemoglobin (Hb) and reducing its affinity for oxygen. Considerable quantitative variability in the levels of DPG and ATP exists in human populations and in a population of hooded (Long-Evans) rats we have studied. This paper presents the results of studies on the genetic component of DPG-level variation in an outbred population of hooded rats. Beginning with about 100 rats, a two-way selection experiment was initiated. Pairs of rats with the highest DPG levels were mated to produce a High-DPG rat strain and animals with the lowest DPG levels were mated to produce a Low-DPG strain. Mean DPG levels responded rapidly to selection and, from generation 3 on, the differences between strain means were highly significant. Ten High-DPG strain rats were intercrossed with 10 Low-DPG strain rats of generation 10 to produce an F₁ generation in which the DPG levels were almost as high as those of High-DPG animals. This indicates partial dominance of High-DPG alleles. The F₂ DPG-level distribution showed two distinct subpopulations. The high DPG subpopulation contained three times as many animals as the low DPG subpopulation. From these results and the statistical analyses performed, it was concluded that the DPG differences between strains were due to an allelic difference at one major locus, the allele carried by the High-DPG strain showing partial dominance over the allele carried by the Low-DPG strain. It appears that this locus may also effect ATP levels to a large extent and is polymorphic in hooded rat populations. Identification of this locus gives us a useful tool for studies of the physiological effects of DPG variability, as well as providing an example of a major gene effect in a quantitatively varying trait.

¹ This work was supported by Public Health Service Training Grant (5-T01-GM-0071), as well as a Michigan Heart Association Grant and the Myers Foundation.
² Present address: Department of Medicine, Harbor General Hospital, Torrance, California 90509.

influences the quantity of oxygen delivered to tissues. Variability among normal individuals in Hb oxygen affinity, brought about by variability in levels of red cell DPG, may result in variable oxygen delivery to the tissues of normal individuals and possibly in variability in the adaptive capacity of the oxygen transport system. Considerable variability in red cell DPG levels exists in normal human populations (Brewer, Oelschlegel and Eaton 1972) and in a population of outbred hooded (Long-Evans) rats we have studied. We are involved in efforts to understand the factors underlying this variability.

Red cell adenosine triphosphate (ATP) is another glycolytic intermediate of importance to the function of the erythrocyte and to oxygen transport. Mature erythrocytes do not produce energy via the Krebs cycle or oxidative phosphorylation. Therefore, ATP generated from glycolysis serves as the primary energy source for these cells. Further, ATP also lowers Hb oxygen affinity when it binds to Hb. The effect of ATP on Hb oxygen affinity, however, is less important than that of DPG because less ATP is present in the red cell. This paper reports the results of a study of the genetic component of variability in both red cell DPG and ATP levels in hooded rats.

**MATERIALS AND METHODS**

*Animal studies*

Long-Evans rats were chosen as the experimental animal because this line has been systematically outbred for more than 40 years (personal communication, Simonsen Laboratories), and, in preliminary studies, these rats showed considerable quantitative variability in red cell DPG levels. A base population of about 100 rats (generation 1), of varying ages, was obtained from Simonsen Laboratories (Gilroy, California). The distribution of DPG levels in these outbred rats was determined and a two-way selection experiment was initiated. Base population rats with the lowest DPG levels were randomly bred, ignoring their sibship origins, to produce a LOW-DPG strain. Similarly, animals with the highest DPG levels were mated to produce a High-DPG strain. This involved a total of 5 high DPG and 5 low DPG matings of generation 1 (base population) animals. In all subsequent matings, in all generations, the rats mated had the highest and the lowest DPG levels of the rats in their generation for the High-DPG and Low-DPG strains respectively. The number of parents and the proportion of each generation chosen as parents varied somewhat from one generation to another.

An intercross study was done using animals from generation 10. Ten pairs, consisting of one High-DPG and one Low-DPG rat were intercrossed. In five of these matings the male was from the Low-DPG strain and in five the male was from the High-DPG strain. The progeny of these matings comprised the F1 generation. When these F1 animals were about 9 weeks old, the males and females of each of the 10 F1 sibships were placed together in a cage for F1 intercross matings to produce the F2 generation.

Eighteen commercially obtained, outbred hooded rats were mated to produce 9 sibships of 93 control rats. This control population served as a sample of the outbred population from which the strains were developed. Properties of the control rats will be assumed to represent those of the original, base population. The actual base population was not used because of the effect of considerable age variation in that population.

*Laboratory studies*

Except for base population animals, where bleeding age varied considerably, blood samples were obtained routinely from all animals at about 6 weeks of age. Each rat was anesthetized
with anhydrous ether and 1 ml of blood was drawn by cardiac puncture into an heparinized
rubinulin syringe with a 26-gauge, ½-inch sterile needle.

For DPG and ATP determinations, 500 µl of well mixed blood was precipitated immediately
after bleeding in 2.0 ml of 6.7% trichloroacetic acid, mixed and placed at -70° until assayed.
Levels of red cell DPG and ATP were determined using the methods of Keitt (1971) and Brewer
and Powell (1966) respectively and were expressed as µmoles per gram Hb. Determinations of
Hb levels were carried out on an aliquot of blood by the standard cyanmethemoglobin method
(Cartwright 1968) and expressed as grams of Hb per 100 ml of whole blood.

RESULTS

(a) Inbreeding estimates: Estimates of the degree of inbreeding were made
using the number of parents producing viable offspring in each generation. The
reciprocal of the average population size, $N_{et}$, for each generation, $t$, or $1/N_{et}$, was
calculated for each strain using the equation:

$$\frac{1}{N_{et}} = \frac{N_f + N_m}{4N_fN_m}$$  \hspace{1cm} (1)*

where $N_f$ and $N_m$ are the number of female and male parents, respectively. The
average rate of inbreeding per generation, $\Delta F$, over 10 generations, was then
computed using the equation:

$$\Delta F = \frac{1}{2t} \left[ \frac{1}{N_{e1}} + \frac{1}{N_{e2}} + \ldots + \frac{1}{N_{e10}} \right]$$  \hspace{1cm} (2)†

Finally, the inbreeding coefficient for generation 10 for each strain was esti-
mated using the relationship:

$$F_t = \Delta F + (1 - \Delta F) \ F_{t-1}$$  \hspace{1cm} (3)‡

The inbreeding coefficient for the initial outbred population was assumed to be
zero.

The average rate of inbreeding over 10 generations was computed to be 0.036
in the High-DPG strain and 0.037 in the Low-DPG strain. The inbreeding
coefficients for generation 10 were estimated to be 0.28 and 0.29 in the High-DPG
and the Low-DPG strains respectively. These inbreeding coefficients estimate
the proportion of heterozygosity present in the original, outbred population,
which has been lost in tenth generation animals.

(b) Response to selection: Figure 1 shows the mean and standard deviation
of DPG levels, the number of progeny in each generation and the mean age in
weeks at which the progeny were bled for each strain and each generation
through generation 10. The rapid response to selection is clearly illustrated. The second
generation of selection produced generation 3 animals in which the High-
DPG strain was significantly $(p < 0.01)$ greater than the Low-DPG strain mean,
although the standard deviations overlapped considerably. This difference was

* Equation (1) is from Falconer (1970) [Equation 4.4].
† Equation (2) is from Falconer (1970) [Equation 4.6 and 4.1 combined].
‡ Equation (3) is from Falconer (1970) [Equation 3.8].
Figure 1.—Means and standard deviations of DPG levels according to generation. The brackets enclose plus or minus one standard deviation. Closed circles (●) represent means of High-DPG animals. Open circles (○) represent mean values from Low-DPG strain rats in each generation. The ** indicates significantly different \((p < 0.01)\) strain means from t-tests. In generation 1, the mean is from about half of the base population. The DPG levels of the other 50 animals were measured using another method and are therefore not strictly comparable.

Maintained throughout subsequent generations. It is relevant to note that the maximum response of DPG levels to selection was attained in very few generations, an expectation if few loci contribute to the difference in mean DPG levels between strains.

The mean age of rats at the time of bleeding for each generation and strain is given at the bottom of Figure 1. This variable is important because DPG levels decline considerably with age. Unfortunately, we did not adopt an identical bleeding schedule until the late generations. This does not affect any of our conclusions but does introduce more variability in DPG levels, making the response to selection less clear. Therefore, where strain mean ages differed considerably for a given generation, as in generations 3 and 4, differences in mean DPG levels between strains may be either increased (generation 4) or decreased (generation 3) because of age differences. Also, the increase in mean DPG levels seen in the High-DPG strain from generation 6 through 8 may be partly explained by the trend for age of bleeding to decrease with generation. Age of bleeding adjustments of the data in Figure 1 were not done. An adequate estimate of the change in DPG level with age, necessary for such adjustments, is difficult to obtain retrospectively because DPG levels are affected by frequency of bleeding and quantity of blood removed as well as by age at time of bleeding. The relative contributions

<table>
<thead>
<tr>
<th>Generation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Progeny</td>
<td>50</td>
<td>59</td>
<td>76</td>
<td>34</td>
<td>65</td>
<td>70</td>
<td>80</td>
<td>57</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Mean age of Bleeding (weeks)</td>
<td>79</td>
<td>80</td>
<td>72</td>
<td>63</td>
<td>6.9</td>
<td>64</td>
<td>6.0</td>
<td>60</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

High-DPG Strain

Low-DPG Strain

An adequate estimate of the change in DPG level with age, necessary for such adjustments, is difficult to obtain retrospectively because DPG levels are affected by frequency of bleeding and quantity of blood removed as well as by age at time of bleeding. The relative contributions
of these factors to the change in DPG levels in repeatedly bled animals are hard to distinguish.

(c) The F₁ and F₂ progeny from intercross of High-DPG and Low-DPG animals: Data on DPG levels in five groups of animals including the F₁ and F₂ are given in Table 1a. The High-DPG and Low-DPG data in Table 1 are each from a group of about 100 strain rats from generations 7, 8 and 9. Data from the 93 Control animals are also given. Note that all animals whose DPG levels are represented in Table 1a were bled at 6 weeks ± 3 days. Therefore, age variability does not confound group contrasts in these data.

Examination of the means of DPG levels in Table 1a reveals that the mean of F₁ animals is more similar to the High-DPG strain mean than to the Low-DPG strain mean. This suggests partial dominance of alleles for High-DPG levels in F₁ animals. Using Sheffe's multiple pairwise contrast method and an experiment-wise error rate of 0.05, we determined that the Low-DPG strain mean DPG level is significantly lower than the other 4 group means in Table 1a. Similarly, the High-DPG strain mean DPG level is significantly greater than the other group means. Only two contrasts, the differences between F₁ and F₂ means and between F₁ and Control means, were not significantly different.

The distributions of DPG levels in the 5 groups of Table 1a are shown in Figure 2. Although the mean of DPG levels for the F₂ animals is very similar to the mean of DPG levels for the F₁ animals (Table 1a), the distributions shown in Figure 2 indicate that, unlike the F₁ population, the F₂ animals fall into 2 subpopulations with respect to DPG levels. The F₂ subpopulation with high DPG levels is about three times as large as the F₂ subpopulation with low DPG levels. This suggests that there is a one-gene difference between the parental strains and that the High-DPG strain carries an allele which is partially dominant over the allele carried by the Low-DPG strain.

If the F₂ distribution is cut into 2 subpopulations at the mid-DPG class with the fewest animals (29 μmoles/g Hb), the 2 subpopulations have 75 and 255

| TABLE 1 |

Descriptive measures in the High-DPG, Low-DPG, Control, F₁, and F₂ rat samples all measured at 6 weeks ± 3 days

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>a) DPG levels (μmoles/g Hb) Mean (Variance)</th>
<th>b) ATP levels (μmoles/g Hb) Mean (Variance)</th>
<th>c) Hb levels (g %) Mean (Variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-DPG</td>
<td>90</td>
<td>34.81 (3.35)</td>
<td>6.45 (0.28)</td>
<td>12.6 (0.98)</td>
</tr>
<tr>
<td>Low-DPG</td>
<td>93</td>
<td>22.40 (4.47)</td>
<td>4.55 (0.44)</td>
<td>13.4 (0.96)</td>
</tr>
<tr>
<td>Control</td>
<td>93</td>
<td>32.02 (4.82)</td>
<td>6.34 (0.77)</td>
<td>12.9 (0.89)</td>
</tr>
<tr>
<td>F₁</td>
<td>85</td>
<td>31.49 (2.07)</td>
<td>6.47 (0.25)</td>
<td>12.8 (0.50)</td>
</tr>
<tr>
<td>F₂</td>
<td>330</td>
<td>31.63 (14.59)</td>
<td>6.20 (0.61)</td>
<td>12.2 (0.51)</td>
</tr>
</tbody>
</table>

F₁ (High-DPG mode) 255 33.45 6.50 12.1
F₂ (Low-DPG mode) 75 25.46 5.20 12.5
FIGURE 2.—Distribution of DPG levels in samples of Control (outbred rats), Low-DPG strain rats, High-DPG strain rats and the F₁ and F₂ populations. All determinations were done on blood drawn from 6-week old animals. Closed arrows indicate means of DPG levels for each group while open arrows indicate means for F₂ subpopulations.

animals. The expectation for a single-locus, two-allele system with dominance (3:1 ratio) is 257.5 and 72.5. The goodness-of-fit Chi-square (with 1 degree of freedom) is 0.1104, far too small to justify rejection of the null hypothesis of a ratio of 3 High-DPG animals to 1 Low-DPG animal. In order to reject the null hypothesis, one would need more than 88 animals or less than 47 animals in the Low-DPG category. The subdivision of F₂ animals is not precisely valid, because with a 3:1 ratio, more genotypically High-DPG animals will be misclassified than genotypically Low-DPG animals. The bimodality is so clear, however, that no other hypothesis fits the distribution as well.

The primary alternative to a single locus accounting for the differences between strains is that the DPG differences between strains are due to multiple,
polymorphic loci. Several analyses were done to determine the most likely of these two possibilities. The results are presented in the Appendix.

(d) **Relationship between DPG and ATP levels:** It was noted early in these studies that red cell ATP levels are positively associated with red cell DPG levels in all hooded rats we have examined. As a result of this association, the selection process which yielded the High-DPG and Low-DPG rat strains also produced High-ATP and Low-ATP strains. Table 1b gives the means and variances of ATP levels of the same groups of animals whose DPG levels are given in Table 1a. High-DPG strain animals, the F1, F2, and Control groups all have quite similar mean ATP levels, while the Low-DPG strain has a greatly reduced mean ATP level. The mean DPG and mean ATP levels of the two F2 subpopulations discussed above are given at the bottom of Table 1. These means indicate that F2 animals with low DPG levels also have low ATP levels and vice versa. Given that both DPG and ATP are products of the same biochemical pathway, glycolysis, the DPG-ATP relationship may well be due to the fact that the DPG-determining locus, by which the strains differ, is also an ATP-determining locus. This possibility requires further testing to be confirmed.

(e) **Hemoglobin levels:** The mean Hb levels of rats in the two strains were consistently different such that genotypically Low-DPG rats had significantly higher Hb levels (Table 1c). Since DPG levels are expressed as μmoles/g Hb, it was possible that the strain differences in DPG levels resulted from differences in the Hb content of the erythrocytes rather than from differences in the cellular DPG concentrations. This possibility was ruled out by finding that the mean cell hemoglobin concentration (MCHC) was not significantly different between strains in a series of about 200 rats of various ages. Therefore, higher Hb levels in Low-DPG rats are probably due to higher red cell counts and this may be a response to inadequate oxygen delivery resulting from low DPG levels.

**DISCUSSION**

The goal of our selection procedure was to produce strains of animals with inherited differences in red cell DPG levels. Our method of two-way selection, including variable numbers of parents contributing to each generation and a variable selection pressure was rather crude compared to many procedures which have been reported in the literature. The genetic cross data are clear, however, making more complex analysis of the selection process unnecessary, for they indicate that the strain differences are likely due to two alleles at one locus.

The choice of an outbred strain as a base population was a good one, for it provided the variability on which selection acted to produce distinct High-DPG and Low-DPG rat populations. Of course, the fact that hooded rats probably have a major gene controlling DPG variability does not exclude the possibility that numerous other loci also contribute to DPG variability. Analysis of the F1 and F2 data suggests only that the High-DPG and Low-DPG strains differ at one locus. If other genes were contributing to the observed strain differences, their effects were very small, for we saw no evidence of them.
Despite the clarity of our results, a number of common problems associated with the identification of major gene effects in quantitative variation are exemplified in this study. For example, when we measured the DPG levels of the 100 rats in the base population, we noted a continuous distribution which was skewed toward low DPG values. Examining the Control rat DPG distribution, shown in Figure 2, this skewing is seen, but the presence of two populations is far more obvious than it was in the base population. We now suspect that a major cause of our inability to detect distinct subpopulations in our base population was due to the confounding effect of age variability on DPG levels. Further, although our assays for DPG, ATP and Hb levels are very accurate, they may vary somewhat over a period of months or years. If we had not produced a large F₂ population (330 animals), drawn blood from them when they were exactly 6 weeks old, and assayed their DPG, ATP and Hb levels over a very short period of time, we may well not have seen such distinct F₂ subpopulations. When quantitative traits are profoundly influenced by environmental factors, as they frequently are, major gene effects are difficult to identify. Very few examples of major genes affecting normal variation in quantitative traits have been reported. When they have been identified, genetic crosses, identification of important environmental effects and careful control of the environmental effects have usually been essential (Spickett, Shire and Stewart 1967; Thoday 1967; Werhahn and Allard 1965).

The principal conclusion from the data presented above is that there is a considerable genetic component in variability in DPG levels in hooded (Long-Evans) rats. Further, our strains, developed by genetic selection for high and low DPG levels, differ at one locus. If we assume that the 93 animals comprising the control group (Figure 2) are representative of the randomly bred Simonsen colony (and our base population), the distribution of their 6 week DPG levels (Figure 2) suggests that the Low-DPG allele is not infrequent in the Simonsen colony. A rough estimate of the Low-DPG gene frequency, based on the fact that 13 of 93 rats in this control population have DPG levels less than 29 μmoles per gm Hb, is 0.37. This strongly suggests that the Simonsen population is polymorphic for this DPG-level determining locus. This locus may also influence red cell ATP levels to a large extent. Preliminary studies on DPG levels in a number of inbred, commercially available rat lines have indicated that a Low-DPG phenotype is present in some and a High-DPG phenotype is present in others of these lines (Noble and Brewer, unpublished observation). Further studies will indicate whether these differences are due to fixation at the locus by which our strains differ.

Biochemical studies involving measurement and comparison of levels of other red cell glycolytic intermediates in the rat strains (Noble and Brewer 1972) and in F₁, F₂ and random rats (unpublished data) strongly suggest that the low DPG and low ATP levels in genotypically Low-DPG rats are the result of low in vivo activity of the red cell glycolytic enzyme, phosphofructokinase. Although we have not yet demonstrated it rigorously, the DPG-level determining locus may be the phosphofructokinase structural gene.
Variability in DPG levels is an important area of study because an individual with high DPG levels may be at an advantage over an individual with lower DPG levels. If all other oxygen transport factors are equal, red cells with higher DPG levels will deliver more oxygen at a given tissue oxygen concentration. The conditions of patients in some chronic disease states (anemia, coronary heart disease) and normal individuals in situations of physical stress (altitude hypoxia, exercise and pregnancy) might be improved with better knowledge of oxygen transport variables. We believe that the data presented in this paper make a general contribution in that they give a clear example of a major gene effect in a continuously distributed trait. Identification of a specific gene affecting variability in red cell DPG levels gives us far more power to investigate the importance of this variability than if we were dealing with a quantitative trait with small contributions from alleles at a number of loci. Work is currently in progress on the physiological and pathological consequences of variation at the DPG locus because DPG levels influence oxygen transport. Already, effects of the DPG-level determining locus on litter size, cholesterol level and possibly rate of learning in F₂ animals indicate that variability in DPG levels may have great physiological importance.

Ultimately, we are interested in DPG variability in human red cells. Considerable variability is present (Brewer, Oelshlegel and Eaton 1972). We hope that the animal studies will give studies of humans direction in pinpointing genetic and environmental effects in DPG variability.

We wish to thank Ed Rothman for his guidance with part of the statistics and Timothy Leonard for his technical assistance.

LITERATURE CITED


The $F_2$ distribution of DPG levels presented in Figure 2 strongly suggests that a single locus with 2 alleles is responsible for the DPG differences between strains. The primary alternative explanation is that multiple, polymorphic loci underlie the differences. We have carried out several analyses to examine which of these explanations is the more probable.

The critical assumption in the analyses below is that 10 generations of selection and inbreeding of about 0.27 are not sufficient to fix a large number of DPG-determining loci. This assumption seems justified because we worked with a relatively small number of generation 1 parents and the strength of selection was not very great. In these analyses, then, our aim is to uncover evidence for heterogeneity remaining in late generation parental strains which would suggest that multiple loci underlie the DPG differences between strains.

Referring to the variances of DPG levels given in Table 1a, when High-DPG strain rats were crossed with Low-DPG strain rates, the $F_1$ produced had a smaller variance than either parental strain. This smaller variance of $F_1$ animals provides evidence for homozygosity of parental strains with respect to DPG-determining loci. Segregation of loci remaining heterozygous in both parental strains, when combined in the $F_2$ animals, would be expected to increase or maintain the variance of DPG levels compared to that in the parental strains; it would not be expected to decrease. If one locus with partial dominance is responsible for the DPG differences, fixation in 10 generations of selection at this level of inbreeding, could easily occur and an $F_2$ variance about the same as, or less than, the parental variance would not be surprising. Thus, in this test, the criterion for the polygenic model is not met.

Two analyses were done to test further the possibility that the DPG differences between parental strains are primarily due to one locus with 2 alleles. First, a model II, random effects

### Table 2

**Analysis of variance tables for $F_1$ and $F_2$ DPG levels**

<table>
<thead>
<tr>
<th>Source</th>
<th>a) Analysis of variance table for $F_1$ DPG levels</th>
<th>b) Analysis of variance table for $F_2$ DPG levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of squares</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>Mean</td>
<td>61951.318</td>
<td>1</td>
</tr>
<tr>
<td>Family</td>
<td>120.478</td>
<td>9</td>
</tr>
<tr>
<td>Sex (Family)</td>
<td>147.341</td>
<td>10</td>
</tr>
<tr>
<td>Error</td>
<td>825.594</td>
<td>65</td>
</tr>
<tr>
<td>Between</td>
<td>705.88</td>
<td>36</td>
</tr>
<tr>
<td>Within</td>
<td>4096.9</td>
<td>293</td>
</tr>
<tr>
<td>Total</td>
<td>4802.8</td>
<td>329</td>
</tr>
</tbody>
</table>

* = Not significant at the 0.05 level of probability.
analysis of variance of $F_1$ DPG levels, with sex nested within families, was done. Table 2a shows the results. The mean squares for family and sex within family are very similar and neither is significantly larger than the variability among animals within sexes. This suggests that the effect of family and of sex within family are small parts of the total variance of $F_1$ DPG levels.

Two important conclusions may be drawn from this analysis. First, the homogeneity of $F_1$ animals suggests that the strain parents mated to produce them were homozygous with respect to DPG-determining alleles. Second, since the parental matings were of two types (high males by low females and low males by high females), if sex-linked loci contributed to the DPG differences between strains, the distribution of DPG levels between sexes within families should be different for the two types of matings. This would be seen as a large sex effect in the analysis of variance. The insignificant effect of sex implies that sex-linked genes probably do not contribute to the DPG differences between strains. The sex-linkage part of this analysis assumes homozygosity for sex-linked loci within parental strains.

The second analysis of family data was a one-way analysis of variance of the animals in the 37 $F_2$ families. This analysis divides the total $F_2$ DPG variance into that within families and that between families. If heterogeneity for DPG-determining loci existed in the parental strains, it would be passed to $F_1$ animals. When $F_1$ families were bred, this heterogeneity would be passed to $F_2$ animals as a result of segregation of loci other than the major gene locus. The $F_2$ families would differ from each other and the between-family variance component would be a large part of the total variance of $F_2$ animals. The results are given in Table 2b. The $F$-test of family differences is not significant at the 5% level of probability, and therefore we fail to reject the hypothesis that no familial differences exist in mean DPG levels. Again, our criterion for the polygenic model is not met.