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Small, inherited differences in blood glucose levels in mice

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1. INTRODUCTION

A number of mutants are known in mice, which have higher blood glucose levels than normal. These are 'diabetes' (Hummel, Dickie & Coleman, 1966), 'obesity' (Mayer, Bates & Dickie, 1951), the yellow agouti mice (Carpenter & Mayer, 1958), and the New Zealand obese mice (Crofford & Davis, 1965). The present paper is an account of some measurements of blood glucose levels in five normal strains of mice, and in crosses between them. The strains were not selected by any criteria related to blood glucose level, but were merely those available.

Each strain of mice appears to be different from the others with respect to genes affecting blood glucose level. The differences are small compared with the effects of the mutants listed above, and it is difficult to determine the genetic basis for these differences.

2. MATERIALS AND METHODS

(i) *Mice*

I shall give a short description of the strains of mice,† because some of their characteristics may be of interest in the light of their different blood glucose levels.

CBA/Cag Cam (*CBA*): sib-mated for at least 50 generations. Coat-colour black-agouti. Fairly active mice, of moderate size.

A/Cam (A): sib-mated for at least 124 generations. Coat-colour white (genotype *bb aa cc*). Tame mice, rather small in size.

SF/Cam (San Fran): sib-mated for over 20 generations. Coat-colour black agouti. They are smaller than CBA or A and rather jumpy.

Peru: bred as a small colony in Cambridge, but not regularly sib-mated. Black agouti in colour. Very small, slim mice, and extremely jumpy.

Swiss: they have been bred as a closed colony, but have been regularly sib-mated for only two years. Coat-colour is white, but backcrosses show that the stock is segregating for a^+ (tan belly), brown (*b*) and dilute (*d*). Large, fat mice, and very placid.

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† All stocks were kept in one room under conditions which were as uniform as possible.

(ii) *Blood glucose determinations*

Samples of blood were taken from the tail, by cutting off about 1 mm of the tip of the tail, and squeezing out a drop of blood. Either 5 or 10 μl of blood were taken, and the number of drops needed to make up the volume varied; the strains of mice differed in the amount of blood which could be obtained, and in the number of drops necessary, but analyses of variance between and within drop number classes gave no evidence that this affected the blood glucose levels.

The mice were sampled when they were about 8 weeks old. There was a small range in their ages, but there was no significant regression of blood glucose on age for these mice. Nor were there significant differences between mice taken first, second, third, etc., from their cages.

The blood glucose was measured by the method described by Bergmeyer & Bernt (1963), using glucose oxidase. The levels of blood glucose were expressed as mg. glucose per 100 ml whole blood.

3. RESULTS

(i) *Means and variances of the strains of mice*

Table 1 shows the means, variances and standard errors of blood glucose levels in mice from the five strains, and from the F_1s with CBA. The strains have similar values, and the distributions for different strains overlap considerably. Distributions constructed of equal numbers of CBA and Peru mice (the two most different strains) are unimodal.

Table 1. *Means, standard errors, and variances of blood glucose levels in the strains of mice and in* F_1*s between CBA and other strains*

	Males			Females		
Stock	Mean ± S.E.	No. of animals	Variance	Mean ± S.E.	No. of animals	Variance
CBA	150·8 ± 1.24	191	292·6	132·1 ± 1·57	158	382·3
Peru	129·8 ± 2·01	133	538·1	119·6 ± 2·21	111	541·0
Swiss	134·4 ± 1·56	168	410·8	129·9 ± 1·34	166	300·2
San Fran	144·1 ± 1·86	148	512·5	138·7 ± 2·21	108	528·8
A	142·8 ± 2·07	90	386·9	128·3 ± 2·52	54	341·4
CBA × Peru	140·4 ± 1·87	110	384·3	128·1 ± 1·86	108	373·4
CBA × Swiss	151·3 ± 1·42	181	365·0	133·1 ± 1·14	223	291·5
CBA × San Fran	146·9 ± 3·14	43	423·2	132·3 ± 3·19	35	356·3
CBA × A	143·1 ± 5·18	20	463·3	122·1 ± 2·78	42	308·0

There are ten possible comparisons between strains; of these, four are significant in both sexes, four in males only, and two in females only. No comparison is non-significant in both sexes. The F_1s with CBA also show significant differences, and thus give further evidence for genetic differences between the strains. Since the mice were kept under uniform conditions and assayed concurrently, these differences must be genetic.

(ii) *Factors affecting blood glucose levels*

(*a*) *Sex differences*

Table 2 gives the results of testing each strain for a sex difference in blood glucose level, together with the percentages of the total variance which can be ascribed to this cause. The sex difference is significant in four of the strains, though the components of variance attributable to sex differences are quite small in two of the strains.

Table 2. *Tests of the sex differences in the strains of mice*

Strain	Difference in mean ♂ − ♀	S.E. of difference	D.F.	t	% of total variance which is due to sex difference
CBA	18·7	1·998	344	9·36***	31·3
Peru	10·2	2·984	242	3·42**	8·5
Swiss	4·5	2·061	332	2·18*	1·8
A	14·5	3·260	142	4·45***	15·0
San Fran	5·4	2·891	254	1·87	0·3

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0·1 % level.

It is possible to test the significance of the variation between strains in the sex difference, as follows: a weighted mean sex difference may be fitted, using the sex differences given in Table 2, and weighting them by the reciprocals of their variances; the sum of squares of residuals, after fitting this mean, is distributed as χ^2. Its value for these data is 25·8, with four degrees of freedom; this is significant at the 1 % level. This result demonstrates that the strains differ in the sex effect on blood glucose levels, as well as in the blood glucose levels themselves.

(*b*) *Components of variance analyses*

Taking each sex separately, I have analysed the total data on the five strains, by means of a components of variance analysis. The variance is partitioned into variance due to the following sources: between strains; between sires within strains; between dams within sires; between litters within dams; within litters. The model for the analysis of variance is

$$y_{ijklm} = \mu + u_i + s_{ij} + d_{ijk} + l_{ijkl} + e_{ijklm},$$

where the y are values for individual mice, μ is the general mean, u, s, d, l and e are effects of strains, sires, dams within sires, litters within dams, and of differences between full-sib littermates of the same sex. Expectations of the mean squares, in terms of components of variance, were found as described by Kempthorne (1957, chapter 13).

The analysis was used to calculate components of variance. Since there are unequal subclass numbers, it is not strictly valid to use variance ratios to test

the significance of the sources of variance, except for between litter, within dam effects. (These effects were significant in all five strains.) The calculated coefficients of the components are, however, quite similar (Table 3) so that the variance ratios may perhaps be used as an indication of significance. On this basis, the only factors contributing significantly to the total variance are differences between strains, and between different litters of the same female. The strain differences account for

Table 3. *Expectations of mean squares, in terms of components of variance, and their coefficients for this data*

	Coefficients of variance components				
Mean squares	σe^2	σl^2	σd^2	σs^2	σu^2
Males					
Strain	1	3·68	6·71	9·77	114·7
Sires	1	3·13	5·02	6·52	—
Dams	1	3·10	4·42	—	—
Litters	1	2·52	—	—	—
Individuals	1	—	—	—	—
Females					
Strain	1	3·25	6·08	8·88	112·4
Sires	1	2·79	4·48	5·79	—
Dams	1	2·63	3·83	—	—
Litters	1	2·59	—	—	—
Individuals	1	—	—	—	—

Definition of variance components: σe^2 individuals within litters; σl^2, litters within dams;, σd^2, dams within sires; σs^2, sires within strains; σu^2, strains.

Table 4. *Components of variance*

(Each component is expressed as a percentage of the sum of all positive components; negative components are taken as zero.)

	Percentage of total variance	
Source	Males	Females
Strain differences	12·5	7·7
Sires within strains	10·2	25·8
Dams within sires	0	0
Litters within dams	34·6	27·7
Individuals within litters	42·7	39·3

only 13 % of the total variance in males, and 8 % in females. Within strains, sex differences and litter differences are the only significant sources of variance. The results of the between-strains analyses are given in Table 4. I have tested the data to see if there are significant differences between mice which are members of a female's first litter, and young born in litters other than the first. Not one of the five strains showed a significant effect in either sex.

These results show that there is a great deal of environmental variation in the blood glucose levels of mice. I have been able to identify one factor which contributes to this: variance between months significantly exceeds variance within

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months (Table 5). These effects are found in all of the strains, and also in nineteen out of the thirty other stocks which were tested. All the strains were assayed over the same period of one year, so that each strain was assayed in each month.

I do not think that these differences are due to experimental error, because, although that might lead to significant differences between months, it would be expected to produce similar changes in all stocks. When the mean blood glucose levels of mice in different strains are plotted against the months in which they were assayed, the strains give strikingly different patterns of change.

Table 5. *Results of analysis for month effects*

(* Denotes significance at the 5 % level, ** at the 1 % level.)

Stock	Sex	F Between months / Within months	D.F. n_1, n_2	P
Strains				
CBA	M	3·17	10, 180	**
	F	7·15	9, 145	**
Peru	M	6·03	7, 125	**
	F	3·36	8, 102	**
Swiss	M	3·09	9, 158	**
	F	3·41	9, 156	**
A	M	1·98	6, 83	—
	F	3·00	4, 49	*
San Fran	M	7·86	10, 137	**
	F	3·67	10, 97	**

(iii) *Results of crosses*

Since there are genetic differences between the strains, segregation of genes should be observed in the F_2s and backcrosses. Assuming that the F_1 variance is due to environmental causes alone, the F_2s and backcrosses should have higher variances than the appropriate F_1s; the additional variance is ascribable to the segregation of genes. For a genetic analysis of blood glucose level to be possible this segregation must be detectable.

Crosses between CBA and each of the other strains were made, and F_2s and backcrosses were bred from the F_1s with Peru, A and Swiss; reciprocal crosses were made in each case, and further backcrosses were made using first and second backcross females from the crosses of CBA with Swiss and with Peru.

In my data, neither the F_2s nor the backcrosses showed significantly different variances from the strains themselves, or from the F_1s between strains. There were no significant correlations between the blood glucose levels of second or third backcross mice and the levels in their first or second backcross mothers. There are three likely reasons for these results.

(*a*) *Genetic variation within the strains.* The Peru and Swiss strains have not been regularly inbred. There are coat-colour genes segregating in the Swiss strain, and there is evidence that genes affecting blood glucose level are also segregating: in this strain regressions of the blood glucose levels of offspring on parents were

significant, and so was the $\frac{1}{2}$-sib correlation in females. There was no evidence for genetic heterogeneity in the other strains, but the Peru strain was not examined in this way.

(*b*) *Environmental variation.* There are significant differences in blood glucose level between mice of the same strain, sex and age. This is true of all the strains, not only those which are not highly inbred; there are therefore environmental effects on blood glucose. The strains' different patterns of seasonal variation suggest that genotype-environment interactions exist.

(*c*) *Multifactorial inheritance.* Although the strain differences are small, it is possible that the strains differ in many genes affecting blood glucose level.

4. DISCUSSION

The results presented above demonstrate the existence of small differences in blood glucose levels, which are genetically determined, although environmental factors contribute considerably to the variation. This conclusion may be summarized by the results of the components of variance analysis of the total data on the five strains, in §3 (ii). The only factors that contribute significantly to the total variance are differences between strains, and between different litters of the same female. Similarly, within the strains, sex differences and litter differences are the only significant sources of variance.

It is interesting to find that a component which contributes only a small proportion to the total variance may nevertheless be significant. If defined genotypes could be made, and replicated, in mice, as they can in *Drosophila*, then it would certainly be possible to analyse the genetic basis of quantitative characters in mice, even characters as difficult as blood glucose levels, in terms of the single genes responsible. No other method is likely to succeed in isolating single genes, because the genetic effects are small in comparison with differences due to environmental effects, so that replication of genotypes is essential.

The approach most likely to give useful information is probably the method of repeated back-crossing together with selection which is described by Wright (1952). Wright shows how this can lead to 'the isolation of one or more relatively important factors, or alternatively the demonstration that there are no such factors'. In the present study, the factors which I have listed in §3(iii) have made this approach unsuccessful, though the mere demonstration that there are genetic differences in blood glucose levels between normal mice is perhaps of interest.

SUMMARY

Blood glucose levels of mice from five strains were measured. The results showed that each strain was different from the others for genes affecting blood glucose level, although the strain differences were small.

Within strains, the chief sources of variation were sex differences, differences between mice from different litters of the same mother, and between mice assayed

in different months. Repeatability of measurements on individual mice was low. Genetic analysis, even by biometrical techniques, was not possible because the small genetic effects were obscured by variation due to other causes.

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