GENETIC VARIATION IN ACTIVITY OF THE ENZYMES OF GLYCOLYSIS AND GLUCONEOGENESIS BETWEEN INBRED STRAINS OF MICE

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ABSTRACT

Variation in the activity of 21 liver and 15 erythrocyte enzymes between seven inbred strains of mice has been studied in a single area of metabolism, glycolysis and gluconeogenesis. Most of the variation between the strains is genetic. From the variation within and between inbred strains heritabilities (H^2) were determined. Out of 35, 26 showed significant values above 0.4. A comparison with previously published work suggests that enzyme activities have mainly dominance and interaction components of variance, and this is discussed in relation to the variation in quantitative characters such as growth. In nine of the pairwise comparisons of the strains, the activity of the enzyme varied more than two-fold. In these cases the genetics and biochemistry of the enzyme was studied; F_2 progeny were produced and assessed for segregation, and the heat stability of the enzyme was determined. No unequivocal segregation was observed, although in one case we found a considerable difference in heat stability. The variations found were not considered to be great enough to be useful as models of human inborn errors of metabolism or to study metabolic control. If such variants are to be found, sources of variation other than inbred strains must be used.

A considerable amount of genetic variation in enzyme activity has been found between inbred strains of mice (YUHAS *et al.* 1967). A number of simple Mendelian variants of enzyme activity have also been found by comparing inbred strains of mice (SEARLE 1977; PAIGEN 1971; RODERICK *et al.* 1971). These are scattered about the metabolic map and few affect enzymes in the same pathway.

We studied the variation in enzyme activity between inbred strains in a single area of metabolism, glycolysis and gluconeogenesis—first, to establish the extent of the variation and whether it is inherited, and second, to determine the variation is inherited in a simple Mendelian manner. We chose this area of metabolism because it was possible to develop semi-automated assays for the enzymes' activity (BULFIELD and MOORE 1974) and because there are a considerable amount of biochemical data on enzymes and intermediates of these pathways

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(NEWSHOLME and GEVERS 1967; SCRUTTON and UTTER 1968; ROLLESTON 1972). Furthermore, there are eleven inherited enzyme deficiencies of erythrocyte glycolysis and two of liver glycolysis known in man (VALENTINE 1968; HARRIS 1975; MCKUSICK 1975).

Any enzyme activity mutants in this area of metabolism can be used to study three major areas: (1) as models in attempting to understand human inborn errors (BLAKE and RUSSELL 1972; KACSER, BULFIELD and WALLACE 1973; BULFIELD and KACSER 1974; GROSS, LONGSHORE and PANGBURN 1975; BULFIELD 1978); (2) to investigate the relationship between structural and regulatory loci (PAIGEN 1971; COLEMAN 1971; PAIGEN *et al.* 1975); and (3) to study the *in vivo* control of biochemical pathways and metabolism (KACSER and BURNS 1968, 1973).

Here we report a survey of the activity of 21 liver and 15 erythrocyte enzymes of carbohydrate metabolism among seven inbred strains.

MATERIALS AND METHODS

Animals: The C57BL/6J, DBA/2J, SWR/J, SM/J and A/J inbred strains were obtained from the Jackson Laboratory, Bar Harbor, Maine. Strain 129/RrJ was obtained from the MRC Laboratory Animals Centre, Carshalton, Surrey, and Peru mice were kindly given by MARGARET E. WALLACE of the Department of Genetics, University of Cambridge. These have been abbreviated in the text to: C57, DBA, SM, SWR, A, 129 and Peru.

Enzyme assays: The preparation of the erythrocyte lysates, liver homogenates and the assays for determination of enzyme activity with the LKB Reaction Rate Analyser, have been described in detail before (BULFIELD and MOORE 1974).

Abbreviations used: AK, adenylate kinase (EC 2.7.4.3); Ald, aldolase (EC 4.1.2.13); ATPase (EC 3.6.1.3); Enol, enolase (EC 4.2.1.11); FDPase, fructose-1, 6-diphosphatase (EC 3.1.3.11); GPUT, galactose-1-phosphate uridyltransferase (EC 2.7.7.10); GAPDH, glyceraldehydephosphate dehydrogenase (EC 1.2.1.12); GK, glucokinase (EC 2.7.1.2); GOPDH, α-glycerophosphate dehydrogenase (EC 1.1.1.8); G6PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); GR, glutathione reductase (EC 1.6.4.2); HK, hexokinase (EC 2.7.1.1); IDH, isocitrate dehydrogenase (EC 1.1.1.41); LDH, lactate dehydrogenase (EC 1.1.1.27/28); PFK, phosphofructokinase (EC 2.7.1.11); PGAM, phosphoglyceraldehyde mutase (EC 5.4.2.1); 6PGDH, 6-phosphogluconate dehydrogenase (EC 1.1.1.43); PGK, phosphoglycerate kinase (EC 2.7.2.3); PHI, phosphohexose isomerase (EC 5.3.1.9); PK, pyruvate kinase (EC 2.7.1.40); TPI, triosephosphate isomerase (EC 5.3.1.1).

RESULTS

Variation in enzyme activity: The activity of 21 liver enzymes in seven strains of mice are shown in Table 1. The largest variation is 8.8 fold between Peru and SM for GAPDH. There are five other enzymes where the variation is greater than two-fold: G6PDH (ratio C57:SWR = 3.7); PK (Peru:SWR = 2.0); Ald (129:Peru = 2.2); GOPDH (A:SM = 2.8) and 6PGDH (C57:SM = 2.2). In all these cases the differences are significant ($P < 0.001$ by the 't' test).

The activity of 15 erythrocyte enzymes in the seven strains are shown in Table 2. Here the largest variation is 6.2 fold between 129 and SWR for Enol. There are three other enzymes where the variation is greater than two-fold:

TABLE 1

Specific activities of 21 liver enzymes in carbohydrates metabolism from seven inbred strains

Strain	C57	DBA	SWR	SM	A	129	Peru
Enzyme							
AK	40.5 ± 1.47	32.0 ± 4.40	26.1 ± 2.55	32.7 ± 3.63	32.6 ± 2.68	28.5 ± 3.40	41.3 ± 5.10
Ald	13.9 ± 1.51	17.0 ± 0.89	12.5 ± 0.73	14.6 ± 2.35	18.4 ± 3.04	22.0 ± 1.21	10.4 ± 1.72
ATPase	2.47 ± 0.048	1.80 ± 0.099	1.47 ± 0.11	1.78 ± 0.034	1.60 ± 0.13	1.53 ± 0.063	2.54 ± 0.10
Enol	36.5 ± 1.64	34.9 ± 2.84	24.3 ± 4.02	26.0 ± 4.35	27.8 ± 2.20	25.9 ± 0.78	34.3 ± 4.00
FDPase	2.59 ± 0.14	2.45 ± 0.14	2.08 ± 0.11	1.81 ± 0.04	1.96 ± 0.07	3.01 ± 0.16	2.40 ± 0.05
GPUT	1.78 ± 0.14	1.52 ± 0.10	1.40 ± 0.08	1.35 ± 0.05	1.53 ± 0.07	1.85 ± 0.08	1.54 ± 0.06
GAPDH	52.6 ± 0.31	55.2 ± 4.50	18.6 ± 4.12	10.9 ± 1.61	50.5 ± 3.52	45.7 ± 6.00	62.8 ± 4.18
GK	0.81 ± 0.063	0.82 ± 0.074	0.67 ± 0.028	0.61 ± 0.049	1.19 ± 0.146	1.18 ± 0.253	0.77 ± 0.054
GOPDH	47.7 ± 2.94	47.2 ± 3.77	37.5 ± 4.65	21.3 ± 1.61	59.5 ± 4.18	36.4 ± 3.42	24.8 ± 3.82
G6PDH	4.02 ± 0.33	1.09 ± 0.095	1.05 ± 0.20	2.76 ± 0.36	2.14 ± 0.13	1.29 ± 0.24	2.52 ± 0.36
GR	5.44 ± 0.76	7.73 ± 0.41	8.34 ± 0.25	8.04 ± 0.12	7.20 ± 0.04	10.18 ± 0.43	7.13 ± 0.83
HK	0.672 ± 0.035	0.407 ± 0.012	0.554 ± 0.038	0.450 ± 0.067	0.532 ± 0.054	0.565 ± 0.026	0.502 ± 0.019
IDH	26.1 ± 1.37	30.4 ± 1.03	28.3 ± 1.40	30.9 ± 2.61	—	—	27.2 ± 2.95
LDH	103 ± 11.4	—	—	117 ± 8.0	105 ± 11.3	95 ± 12.0	94 ± 5.5
PFK	8.02 ± 0.15	7.13 ± 0.18	6.97 ± 0.04	7.36 ± 0.20	—	—	7.34 ± 0.57
PGAM	37.6 ± 4.65	48.1 ± 0.68	30.5 ± 1.87	40.1 ± 2.26	49.5 ± 3.29	35.4 ± 2.26	38.2 ± 1.64
6PGDH	2.78 ± 0.25	1.70 ± 0.18	1.72 ± 0.09	1.27 ± 0.20	1.93 ± 0.12	1.92 ± 0.06	1.39 ± 0.15
PGK	172 ± 8.8	171 ± 8.9	143 ± 7.3	141 ± 3.6	157 ± 8.4	145 ± 13.6	153 ± 13.1
PHI	89.0 ± 18.8	88.7 ± 26.7	79.1 ± 4.6	88.8 ± 11.5	86.6 ± 22.4	77.0 ± 4.7	82.3 ± 29.2
PK	104 ± 5.2	84.6 ± 3.4	51.3 ± 2.7	65.8 ± 5.2	68.0 ± 4.4	64.7 ± 5.0	105 ± 8.3
TPI	723 ± 40	725 ± 40	745 ± 76	717 ± 45	712 ± 24	609 ± 41	699 ± 27

Enzyme activities expressed as μmoles/min/g wet tissue; means ± standard error of the mean, $n = 6$.
Abbreviations listed in MATERIALS AND METHODS.

TABLE 2

Specific activities of 15 erythrocyte enzymes in carbohydrate metabolism for seven inbred strains

Strain	C57	DBA	SWR	SM	A	129	Peru
Enzyme							
AK	0.74 ± 0.13	0.94 ± 0.04	0.74 ± 0.05	0.89 ± 0.04	1.10 ± 0.12	0.90 ± 0.06	0.95 ± 0.04
Ald	0.55 ± 0.04	0.85 ± 0.02	0.91 ± 0.01	0.75 ± 0.03	0.88 ± 0.06	0.63 ± 0.09	0.61 ± 0.05
ATPase	0.50 ± 0.01	0.75 ± 0.03	0.52 ± 0.04	0.38 ± 0.02	—	—	0.38 ± 0.02
Enol	2.28 ± 0.16	3.33 ± 0.27	0.60 ± 0.07	0.90 ± 0.15	3.79 ± 0.18	2.61 ± 0.16	3.69 ± 0.33
GAPDH	2.95 ± 0.51	4.67 ± 0.83	4.67 ± 0.41	6.65 ± 0.48	7.33 ± 0.25	6.30 ± 1.34	3.83 ± 0.18
G6PDH	3.31 ± 0.61	4.17 ± 0.15	4.91 ± 0.17	4.27 ± 0.17	5.26 ± 0.38	4.95 ± 0.27	4.68 ± 0.43
GR	1.26 ± 0.06	1.55 ± 0.08	2.81 ± 0.08	1.46 ± 0.06	2.13 ± 0.05	1.58 ± 0.05	1.62 ± 0.06
HK	0.49 ± 0.04	0.51 ± 0.03	0.72 ± 0.03	0.63 ± 0.04	0.71 ± 0.03	0.54 ± 0.02	0.54 ± 0.03
PFK	4.63 ± 0.22	4.38 ± 0.09	4.96 ± 0.22	4.77 ± 0.13	—	—	4.03 ± 0.11
PGAM	2.51 ± 0.16	3.19 ± 0.13	2.12 ± 0.06	3.19 ± 0.04	3.35 ± 0.12	2.37 ± 0.07	3.14 ± 0.19
6PGDH	0.84 ± 0.04	0.83 ± 0.04	1.15 ± 0.03	1.20 ± 0.13	1.38 ± 0.01	1.22 ± 0.03	1.14 ± 0.05
PGK	8.1 ± 0.53	16.4 ± 2.09	10.5 ± 1.59	11.4 ± 0.17	15.7 ± 0.61	14.1 ± 0.56	14.9 ± 0.62
PHI	14.7 ± 0.57	20.2 ± 0.33	17.4 ± 0.20	20.8 ± 0.89	19.3 ± 0.82	17.9 ± 1.25	18.0 ± 0.23
PK	14.3 ± 1.73	15.7 ± 0.41	13.7 ± 0.39	16.6 ± 0.63	18.4 ± 1.29	16.8 ± 0.77	18.0 ± 1.12
TPI	107 ± 1.4	110 ± 10.0	114 ± 4.3	131 ± 7.6	123 ± 5.4	124 ± 10.0	111 ± 5.7

Enzyme activities expressed as μmoles/min/ml RBC, means ± standard error of the mean, $n = 6$.

GAPDH (ratio A:C57 = 2.4); PGK (DBA:C57 = 2.0) and GR (SWR:C57 = 2.2). All these differences are significant ($P < 0.001$).

Degree of genetic determination (H^2): Using the data from Tables 1 and 2, the degree of genetic determination (or heritability in the broad sense, H^2) was calculated from the variance within and between the strains C57, DBA, SWR, and SM for each enzyme (FALCONER 1960; YUHAS *et al.* 1967). The H^2 values are presented in Table 3, those for erythrocyte enzymes being in most cases higher than those for liver enzymes.

Genetic and biochemical analysis of the enzyme variation: The variation in enzyme activity between C57, DBA, SM and SWR was subjected to initial analysis in two ways. (1) Where a pair of strains differed greater than two-fold in the activity of an enzyme, they were crossed, and the F_2 animals were assayed for the activity of the enzyme and the data examined for Mendelian segregation. (2) In addition heat stability studies were performed on the enzyme extracted from the two parental strains to determine whether there was any structural alteration in the enzyme. These results are shown in Table 4.

Only in the case of liver GAPDH was there a significant difference in heat stability between a pair of strains. No F_2 segregation was demonstrated for four

TABLE 3

Degrees of genetic determination (broad sense heritabilities-H^2) for 20 liver enzymes and 15 erythrocyte enzymes†

Enzyme	H^2‡ liver	P*	H^2‡ erythrocyte	P*
AK	0.320	NS	0.172	NS
Ald	−0.044	NS	0.859	<0.001
ATPase	0.627	<0.05	0.929	<0.001
Enol	0.433	NS	0.949	<0.001
FDPase	0.391	<0.05	—	—
GPUT	0.357	NS	—	—
GAPDH	0.615	<0.05	0.788	<0.01
GK	0.583	<0.05	—	—
GOPDH	0.706	<0.01	—	—
G6PDH	0.412	NS	0.851	<0.01
GR	0.746	<0.05	0.970	<0.001
HK	0.568	<0.05	0.654	<0.05
IDH	0.111	NS	—	—
PFK	0.148	NS	0.486	NS
PGAM	0.626	<0.05	0.885	<0.01
6PGDH	0.785	<0.01	0.704	<0.05
PGK	0.462	<0.05	0.526	<0.05
PHI	−0.410	NS	0.889	<0.01
PK	0.845	<0.05	0.134	NS
TPI	−0.234	NS	0.240	NS

* Significance by the *F* test.
† See MATERIALS AND METHODS for abbreviations.
‡ Heritabilities calculated after YUHAS *et al.* 1967.

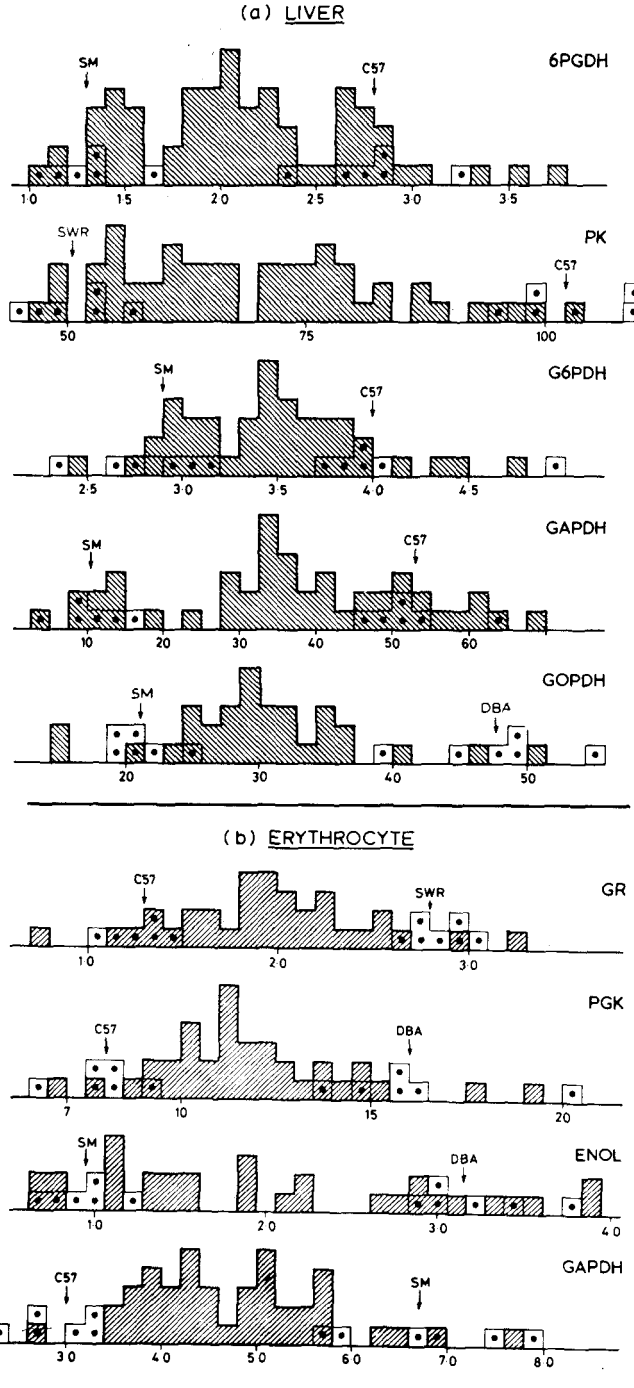

FIGURE 1.—Enzyme activity distribution of F_2's and parental strains. Only differences which were greater than two-fold in the parental strain are shown. See MATERIALS AND METHODS for

TABLE 4

*Heat stability of enzymes where strain differences in activity are greater than two fold in the liver and erythrocytes**

	Enzyme	Strains	Mean activity (from Tables 1, 2)	Heat stability 30 mins at X°	Heat stability Percent activity left
Liver	6PGDH	C57	2.8	52°	65
		SM	1.3		58
	PK	C57	104	45°	48
		SWR	51		47
	G6PDH	C57	4.0	37°	48
		SM	2.8		61
	GAPDH	C57	53	36°	43
		SM	11		42
	GOPDH	DBA	47	too	—
		SM	21	unstable	
Erythrocyte	GR	SWR	2.8	too stable	—
		C57	1.3	(clotted)	
	PGK	DBA	16	46°	48
		C57	8		49
	ENOL	DBA	3.3	39°	58
		SM	0.9		62
	GAPDH	SM	6.7	36°	38
		C57	3.0		0

All heat stability determinations are the means of six animals, each repeated three times. Only erythrocyte GAPDH significantly differs between the strains.

Before the heat stability tests the homogenates were dialysed overnight at 0–5° against 500 volumes of 0.05M Tris pH 7.4 and 1 mM dithiothreitol (Sigma).

* See MATERIALS AND METHODS for abbreviations.

enzymes, although there was, possibly, segregation for liver G6PDH and erythrocyte GAPDH and Enol and probably for liver 6PGDH and GAPDH (Figure 1).

DISCUSSION

Our survey of 36 enzymes among seven strains of mice indicates that, while there is a lot of variation in enzyme activity, only in nine cases is it higher than two-fold. About 20 variations in enzyme activity have been claimed to be due to single-locus differences (SEARLE 1977). Most of these variants show about a two to three-fold difference in enzyme activity. In five cases the activity differences are much larger [proline oxidase, 20-fold (BLAKE 1972); phosphorylase kinase, 100-fold (LYON 1970; GROSS, LONGSHORE and PANGBURN 1975); histidase, 20-fold (KACSER, BULFIELD and WALLACE 1973); catalase, 100-fold (FEIN-

abbreviations used. The hatched histograms represent the F_2 results; the dotted histograms the parental strains with arrows showing the means. The abscissae in (a) are enzyme activities in μmoles/min/g wet weight liver at 30° and in (b) are enzyme activities in μmoles/min/ml erythrocytes at 30°. The ordinates are the number of animals; each square represents one animal.

STEIN *et al.* 1966); tyrosinase, 20-fold (COLEMAN 1962)]. In spite of these large differences in enzyme activity, there is no apparent effect on viability in any of these mutants. Large enzyme differences between inbred strains are therefore not necessarily excluded. It must be stressed, however, that the human inborn errors homologous to the five mentioned above have only minor clinical symptoms (MCKUSICK 1975).

Segregational analysis has been used by several workers to assign variation between inbred strains to a single locus. However, when the variation is relatively small, as in the present case (Figure 1), the power of analysis is reduced [Gpd-1 and Gpd-2 (HUTTON 1971); Gdc-1 (KOZAK 1972)]. It is therefore difficult to assign variation unequivocally to a single locus in any case (Figure 1). Recombinant inbred lines (SWANK and BAILEY 1973) and a complex biometrical technique (STEWART and ELSTON 1973) were developed in an attempt to deal with this problem. Another approach is to determine the Michaelis constants, electrophoretic mobility, inhibition constants or heat stability of the enzymes in the original strains (PAIGEN 1971; HUTTON 1971; KOZAK 1972; SHEPPARD, ALBERSHEIM and MCCLEARN 1970; COLEMAN 1971; SANNO, HOLZER and SCHIMKE 1970). Over half the alleles at the structural loci for G6PDH and hemoglobin in man differ in heat stability (LEHMANN and CARRELL 1969; PAIGEN 1971; YOSHIDA, BEUTLER and MOTULSKY 1971). By this criterion, however, only one out of the nine enzymes (varying greater than two-fold between our inbred strains) is controlled by segregation at a single structural locus (Table 4, erythrocyte GAPDH). This indicates that the magnitude of the variation between inbred strains is small, and that it may not be due to segregation at a single locus in each case. It is worth noting that one of the enzymes (glutathione reductase; (GR) displayed in the erythrocyte segregation data (Figure 1) has been recently reported to have different kidney electrophoretic alleles in the parental strains (NICHOLS and RUDDLE 1975). We are investigating whether this kidney variation is associated with the erythrocyte variation between these two strains. It is possible that differences in erythrocyte enzyme activities might be caused by lability differences due to differential erythrocyte aging and therefore differences in recticulocyte count. Ranking the strains for enzyme activity is random over the 15 erythrocyte enzymes, except for C57 whose enzymes' activities are usually lower than the others. We have not tested whether this phenomenon in C57 is due to a red blood cell age profile. However, published data suggest that C57 mice do not differ from DBA and several other strains in red blood cell count, percent haematocrit or recticulocyte count (RUSSELL, NEUFELD and HIGGINS 1951).

Although it cannot be ruled out that some of the variation is due to differences at the structural locus some is probably due to differences at loci that affect either the rate of synthesis or of degradation of the enzymes (PAIGEN 1971). Similarly, because crude tissue homogenates were used, differences at loci affecting the activity of the enzymes through the concentrations of co-factors, inhibitors or activators might also be detected. All these molecular influences are, by their

nature, themselves subject to the influence of many loci. Finding small quantitative differences in enzyme activity between inbred strains, which is difficult to assign to segregation at a single locus, is not surprising.

Enzyme activity can be treated as a quantitative character, even if occasional segregation at a major locus is demonstrated. The variation between inbred strains can be subjected to an analysis of variance. This enables us to calculate a broad sense heritability, H^2—Table 3 (FALCONER 1960; YUHAS *et al.* 1967). These H^2 values are high (of the order of 0.5; see also YUHAS *et al.* 1967), which contrasts with very low narrow sense heritabilities h^2 (FALCONER 1960), determined from parent-offspring regression of random bred mice (of the order of zero; BULFIELD and WALKER 1974), whereas ten-week body weight is high in both cases ($H^2 = 0.68$, $h^2 = 0.40$). This suggests that while body weight has a large additive genetic component, enzyme activities have mainly dominance and interaction components of variance.

We set out to discover enzyme variants in glycolysis and gluconeogenesis that could be used as models of inborn errors in man and to investigate the control of metabolism and development. While variation was discovered between inbred strains, it is not sufficiently large to be useful.

Differences in extracted activities of crude homogenates are likely to reflect *in vivo* differences. This is suggested by those cases where enzyme determinations are accompanied by measurement of the *in vivo* metabolic consequences on substrates and products (COLEMAN 1962; BLAKE 1972; KACSER, BULFIELD and WALLACE 1973). In these investigations, the pool movements and changes in flux are, at least qualitatively, in agreement with the extracted enzyme activity differences. It is therefore likely that the observed enzyme differences, albeit small ones, will correspond to small differences in the metabolic pattern of the strains. Our findings of such variation, together with our conclusion as to its multigenic origin, suggests a basis for the molecular nature of metric characters. Electrophoretic variants unaccompanied by demonstrable activity differences, by contrast, are only exceptionally likely to have significant effects on the metabolism.

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