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Pleiotropic and Other Genetic Effects Influencing the Activities of Brain and Liver Enzymes in Congenic Lines of C57BL/6J Mice with Defined Electrophoretic Variant Markers

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A single genetic factor may affect the realization of several enzymes. To investigate the extent of pattern pleiotropy in the mouse, the activities of 28 enzymes in livers and brains from an inbred stock of C57BL/6JNctr and five F_1 stocks heterozygous for known electrophoretic variants were measured. Five congenic backcross stocks of C57BL/6J, each homozygous for one or more electrophoretic markers, were mated with C57BL/6JNctr to construct the heterozygous variant F_1 stocks. One of the five F_1 stocks had no enzyme activities significantly different from those of C57BL/6JNctr, while two had one enzyme, one had four enzymes, and another had six enzymes with activities that were significantly different from those of C57BL/6JNctr. The latter two F_1 stocks with multiple activity differences were those having the largest proportion of their genome of donor origin. Two of the F_1 stocks were different from each other for one enzyme, and two were different for another enzyme. These differences and the relationship of these enzyme activities to the variant genes suggest that several genetic factors may affect an enzyme's realization.

KEY WORDS: enzymes; congenic; pleiotropy; genetic factor.

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INTRODUCTION

The expression of an enzyme's ultimate phenotype is dependent upon a complex set of processes involved in its realization (Paigen, 1971). Genetic control over these processes by structural, regulatory, temporal, or processing genes has been described and reviewed (Paigen, 1979). This suggests that multiple genetic factors influence the final expression of a realized enzyme. Modification of any one of these factors could result in alteration of that enzyme's activity.

A single genetic factor may also affect the realization of several enzymes. For example, posttransplantational sialylation of several acid hydrolases (Lally and Shows, 1977; Womack and Eicher, 1977; Dizik and Elliot, 1978), expressed as changes in enzyme activity, has been attributed to pleiotropic effects of a single neurominidase (Neu-1) gene (Womack *et al.*, 1981). If such effects play a role in the realization of many enzymes, then most defined structural gene alterations might be expected to modify the activity of other enzymes.

A backcross project has made available 13 stocks of C57BL/6J mice which carry alterations of defined structural genes (Roderick and Womack, 1981). The influence which a heterozygous variant genome of C57BL/6J has upon the activities of 28 brain and liver enzymes, relative to their activities in inbred C57BL/6JNctr mice, has been investigated using 5 of these stocks.

MATERIALS AND METHODS

An inbred stock of C57BL/6JNctr and five congenic backcross stocks of C57BL/6J, designated BC2, BC6A, BC7, BC10, and BC13, were used in this study (Table I). The allelic variants of the congenic line of BC2 were agouti

Table I. Allelic Variants on C57BL/6J Background

Line	Locus[a]	Chromosome	C57BL/6J allele	New allele	Strain of origin
BC2	*a*	2	*a*	a^+	CE/J
	Amy-1	3	*a*	*b*	CE/J
	Amy-2	3	*a*	*b*	CE/J
BC6A	*Gdc-1*	15	*b*	*d*	Castaneus
BC7	*Mor-1*	5	*a*	*b*	MOR stock
BC10	*Es-10*	14	*a*	*c*	Molossinus
	Np-1	14	*a*	*b*	Molossinus
BC13	*Es-8*	7	*a*	*b*	Castaneus

[a] *Amy-1* and *-2*, amylase genes; *Gdc-1*, glyceraldehyde decarboxylase gene; *Mor-1:* Malate dehydrogenase gene; *Es-8 and -10:* Esterase genes; *Np-1:* Nucleoside phosphorylase gene.

(a^+) and two amylases (*Amy-1*^{b} and *Amy-2*^{b}). All were derived from CE/J. The variants in line BC10 were an esterase (*Es-10*^{c}) and a nucleoside phosphorylase (*Np-1*^{b}), both derived from the interfertile subspecies *Mus musculus molossinus*. The variant in congenic line BC6A was glycerol phosphate dehydratase (*Gdc-1*^{d}), and the variant in BC13 was another esterase (*Es-8*^{b}), each from another interfertile subspecies, *Mus musculus castaneus*. The variant in BC7 was mitochondrial malate dehydrogenase (*Mor-1*^{b}) from the MOR stock originally isolated from *Mus musculus molossinus*. Each marker, supposedly having arisen as a spontaneous mutation in the donor stock, was individually transferred to the C57BL/6J background by backcrossing through at least nine generations. For ease of maintenance, the last backcross generation of each congenic stock was intercrossed to make the respective congenic strains homozygous for the variant markers.

Homozygous mice from each of the five congenic stocks were mated to C57BL/6JNctr mice when they became 6 weeks of age, to produce approximately 50 F_1 heterozygotes per stock (designated $BC2F_1$, $BC6AF_1$, $BC7F_1$, $BC10F_1$, and $BC13F_1$). At the same time, matings of C57BL/6JNctr mice were established to produce 50 males and 50 females of the inbred stock. All matings provided 5–10 individuals from each group born within a given week. The mice were randomly distributed among seven experimental blocks in such a way that each block was evenly represented by an equal ratio of mice from each stock. These blocks became sacrifice, tissue preparation, and enzyme activity analysis units. This design allowed for comparisons of enzyme activities across and within the blocks, eliminating differences which might result from sacrifice, tissue preparation, or analysis phenomena. Thus, the potential for identification of relatively small activity differences was maximized.

When mice became 10 weeks old, cages containing three to five mice were retrieved individually from the animal room with care taken to minimize disturbance of remaining animals. All mice within a particular cage were decapitated in an adjacent room within 10–15 sec. Brain and liver tissues were surgically removed, wrapped in aluminum foil, and frozen in liquid nitrogen. All mice within a block were killed in a similar manner within a 60-min period (1:00–2:00 pm CST). Tissues were retrieved from the liquid nitrogen and stored at −70°C to await preparation and analysis. Tissue preparations and analyses were performed by blocks according to our standard procedures (Feuers *et al.*, 1980). The enzymes analyzed from brain were adenylate kinase (AK), pyruvate kinase (PK), malate dehydrogenase (MDH), adenosine triphosphate (ATPase), creatine phosphokinase (CPK), phosphoglucose isomerase (PGI), creatininase (CR), and succinate thiokinase (STK). The enzymes analyzed in the liver were glutamate oxaloacetate transaminase

(GOT), citrate cleavage enzyme (CCE), isocitrate dehydrogenase (ICD), glutathione reductase (GR), glycerokinase (GlK), glutamate dehydrogenase (GlDH), cytochrome *c* reductase (CCR), glyoxalate reductase (GlyR), alanine aminotransferase (GPT), amino acid oxidase (AAO), serine dehydratase (SDH), sorbitol dehydrogenase (SbDH), alcohol dehydrogenase (ADH), malic enzyme (ME), fatty acid synthetase (FAS), fructose diphosphatase (FDPase), lactate dehydrogenase (LDH), fructose diphosphate aldolase (FDP,Ald), fructose-1-phosphate aldolase (F1P,Ald), and pyruvate decarboxylase (PDC).

The activity data were sorted by computer from coded entry and collated according to block, variant stock, and sex. Mean activities, standard deviations, and coefficients of variation (CV) were calculated. A one-way analysis of variance was performed for each sex and enzyme, and differences were delineated using Duncan's multiple-range test (Winer, 1971). A difference between two stocks was considered significant if the analyses of variance were significant at the 0.1 level in both sexes for the enzyme. Since these analyses of variance for each sex are independent, this criterion results in a significance level for stock differences of ≤ 0.01 for the enzyme.

Partial correlation coefficients corrected for sex and strain were calculated (Kshirsagar, 1972). A factor analysis (Seal, 1968) was performed for those multiple enzyme differences observed within stocks to estimate the minimum number of factors which could account for any observed partial correlations.

RESULTS

Thus far we have examined the activities of 28 enzymes in stocks of C57BL/6JNctr mice and F_1 mice produced from matings of this inbred with 5 of the 13 available congenic lines. The mean activities and CVs for all enzymes for each stock are listed in Table II. The CVs for C57BL/6JNctr mice are relatively low and range from 0.02 for CPK in males to 0.39 for SDH in females. Only AAO (0.38, females; 0.37, males), CCE (0.32, females), SDH (0.39, females), and STK (0.27, females) have CV values above 25%. The CVs for enzymes measured in the F_1 stocks are similar to those of C57BL/6JNctr.

Many statistically significant differences among enzyme activities were obtained (Table II). Although most of these involved differences in only one sex, several differences were below the required 0.1 level for both sexes. In fact, of those with differences for both sexes, only AAO males ($P = 0.06$) and GlK females ($P = 0.06$) were above 0.05. Differences among stocks were found for the enzymes AAO, GlDH, GlK, F1P,Ald, ATPase, CR, MDH, STK, and FDP,Ald (Table III). The activities of CR were different in

Table II. Comparisons of Mean Enzyme Activities Among Stocks[a,b]

Brain enzyme	Sex	C57BL/6JNctr act (cv)	BC10F$_1$ act (cv)	BC13F$_1$ act (cv)	BC2F$_1$ act (cv)	BC6AF$_1$ act (cv)	BC7F$_1$ act (cv)
AK	F	4731 (.07)	4940 (.09)	4802 (.07)	4814 (.12)	4834 (.06)	4645 (.05)
	M	4731 (.07)	4877 (.05)	4949 (.06)	4842 (.09)	4643 (.11)	4697 (.05)
ATPase	F	460 (.17)^{c}	**544** (.16)^{a}	496 (.17)^{bc}	499 (.16)^{abc}	509 (.14)^{ab}	466 (.15)^{bc}
	M	448 (.19)^{c}	**542** (.16)^{a}	518 (.18)^{ab}	516 (.17)^{ab}	474 (.15)^{bc}	487 (.15)^{bc}
CPK	F	1692 (.03)	1691 (.02)	1693 (.02)	1681 (.02)	1702 (.03)	1708 (.03)
	M	1695 (.02)	1697 (.03)	1710 (.03)	1697 (.03)	1687 (.03)	1703 (.02)
CR	F	602 (.20)^{b}	**684** (.15)^{a}	602 (.17)^{b}	625 (.23)^{ab}	684 (.14)^{a}	585 (.15)^{b}
	M	617 (.15)^{c}	**675** (.13)^{ab}	707 (.16)^{a}	683 (.16)^{ab}	634 (.16)^{bc}	632 (.16)^{bc}
MDH	F	33608 (.09)^{b}	**35744** (.06)^{a}	34366 (.08)^{ab}	33520 (.08)^{b}	35291 (.08)^{a}	33468 (.05)^{b}
	M	32836 (.09)^{c}	**35560** (.05)^{a}	35580 (.07)^{a}	34399 (.08)^{ab}	33624 (.11)^{bc}	34378 (.05)^{ab}
PGI	F	4237 (.03)	4275 (.03)	4260 (.03)	4253 (.03)	4270 (.02)	4241 (.03)
	M	4224 (.04)	4280 (.02)	4298 (.03)	4248 (.04)	4229 (.04)	4249 (.02)
PK	F	14359 (.07)	14367 (.06)	14417 (.08)	14058 (.09)	14546 (.07)	13947 (.06)
	M	13988 (.11)	14229 (.05)	14452 (.06)	14263 (.09)	14006 (.10)	14110 (.05)
STK	F	750 (.27)^{c}	**942** (.18)^{a}	798 (.27)^{bc}	788 (.22)^{bc}	860 (.20)^{ab}	760 (.20)^{bc}
	M	747 (.23)^{d}	**939** (.21)^{a}	878 (.24)^{ab}	877 (.23)^{abc}	789 (.18)^{bcd}	766 (.21)^{cd}
Liver Enzyme							
AAO	F	273 (.38)^{b}	314 (.27)^{ab}	307 (.21)^{ab}	**352** (.36)^{a}	304 (.28)^{ab}	310 (.26)^{ab}
	M	217 (.37)^{b}	249 (.25)^{ab}	260 (.23)^{ab}	**267** (.38)^{a}	255 (.33)^{ab}	261 (.20)^{ab}
ADH	F	198 (.13)	203 (.09)	193 (.10)	190 (.10)	194 (.13)	198 (.20)
	M	169 (.09)^{a}	173 (.09)^{a}	170 (.09)^{a}	151 (.12)^{b}	165 (.10)^{a}	170 (.12)^{a}
CCE	F	285 (.32)	294 (.18)	299 (.17)	308 (.32)	294 (.27)	287 (.24)
	M	232 (.25)	258 (.25)	271 (.19)	245 (.25)	254 (.27)	268 (.22)
CCR	F	1331 (.18)^{a}	1280 (.09)^{a}	1248 (.12)^{ab}	1153 (.11)^{b}	1270 (.16)^{a}	1241 (.19)^{ab}
	M	904 (.13)	868 (.24)	901 (.17)	928 (.19)	932 (.29)	846 (.15)
FAS	F	261 (.09)	266 (.10)	263 (.10)	251 (.09)	259 (.09)	264 (.10)
	M	275 (.08)^{bc}	282 (.08)^{ab}	282 (.09)^{ab}	263 (.11)^{c}	272 (.08)^{bc}	288 (.09)^{a}
FDPase	F	175 (.14)	179 (.11)	178 (.13)	172 (.12)	178 (.11)	179 (.11)
	M	142 (.10)	149 (.11)	153 (.13)	141 (.20)	145 (.13)	147 (.14)
FDP. Ald	F	364 (.15)^{a}	**325** (.19)^{b}	343 (.19)^{ab}	**314** (.20)^{b}	343 (.16)^{ab}	347 (.17)^{ab}
	M	389 (.14)^{a}	**352** (.20)^{bc}	379 (.14)^{ab}	**339** (.21)^{c}	373 (.11)^{abc}	377 (.14)^{ab}
FIP. Ald	F	933 (.08)	896 (.08)	909 (.07)	906 (.08)	922 (.07)	919 (.07)
	M	976 (.08)^{a}	928 (.09)^{b}	942 (.06)^{ab}	922 (.09)^{b}	942 (.06)^{ab}	942 (.07)^{ab}
GIDH	F	2033 (.16)^{b}	**2296** (.15)^{a}	**2238** (.11)^{a}	**2315** (.20)^{a}	2057 (.15)^{b}	**2266** (.12)^{a}
	M	1661 (.15)^{b}	**1949** (.11)^{a}	**1915** (.13)^{a}	**1962** (.18)^{a}	1824 (.14)^{a}	**1948** (.12)^{a}
Glk	F	440 (.24)^{b}	497 (.25)^{a}	486 (.21)^{a}	**516** (.28)^{a}	466 (.22)^{ab}	476 (.20)^{ab}
	M	380 (.21)^{b}	431 (.20)^{a}	445 (.20)^{a}	**437** (.26)^{a}	417 (.22)^{ab}	428 (.26)^{ab}
GlyR	F	16716 (.08)	16538 (.06)	16406 (.08)	16245 (.06)	16448 (.06)	16256 (.05)
	M	17627 (.07)	17253 (.08)	17380 (.09)	17175 (.06)	17815 (.07)	18053 (.08)
GOT	F	11216 (.09)	11712 (.08)	11519 (.11)	11756 (.11)	11451 (.09)	11776 (.10)
	M	9355 (.09)	10004 (.11)	10552 (.11)	10108 (.12)	10129 (.19)	10667 (.14)
GPT	F	2527 (.10)	2480 (.11)	2489 (.13)	2473 (.13)	2453 (.13)	2522 (.12)
	M	2161 (.09)	2117 (.09)	2236 (.13)	2088 (.16)	2152 (.13)	2182 (.09)
GR	F	503 (.07)	502 (.04)	493 (.09)	494 (.06)	494 (.06)	496 (.08)
	M	550 (.08)^{b}	565 (.06)^{ab}	562 (.06)^{ab}	539 (.09)^{b}	547 (.08)^{b}	576 (.09)^{a}
ICD	F	3342 (.10)	3353 (.10)	3330 (.11)	3330 (.11)	3205 (.14)	3379 (.11)
	M	3149 (.13)^{a}	3108 (.14)^{ab}	3236 (.10)^{a}	2923 (.13)^{b}	3086 (.10)^{ab}	3321 (.15)^{a}
LDH	F	22847 (.13)	22454 (.15)	21853 (.15)	21804 (.11)	21859 (.13)	22193 (.14)
	M	24268 (.09)^{a}	24147 (.14)^{a}	23806 (.14)^{ab}	22319 (.11)^{b}	24209 (.11)^{a}	25159 (.13)^{a}
ME	F	323 (.21)	344 (.23)	334 (.20)	312 (.20)^{c}	301 (.27)	331 (.22)
	M	359 (.25)^{a}	399 (.25)^{a}	372 (.25)^{a}	286 (.39)^{c}	301 (.33)^{bc}	348 (.21)^{ab}
PDC	F	10841 (.10)	10620 (.10)	10685 (.11)	10613 (.09)	10565 (.09)	10616 (.09)
	M	11324 (.06)	11255 (.08)	11300 (.09)	10949 (.10)	11224 (.07)	11746 (.08)
SbDH	F	2002 (.09)	1952 (.09)	1931 (.11)	1913 (.13)	1989 (.10)	1976 (.11)
	M	1887 (.09)^{a}	1931 (.11)^{a}	1900 (.08)^{a}	1744 (.11)^{b}	1772 (.09)^{b}	1846 (.09)^{ab}
SDH	F	630 (.39)	556 (.21)	613 (.24)	596 (.36)	616 (.44)	646 (.23)
	M	404 (.18)	413 (.21)	438 (.31)	451 (.39)	478 (.48)	469 (.22)

[a] Activity (Act) = μmoles NAD^+/h/g tissue; Coefficient of variation (cv) = standard deviation/mean activity a, b, c, d similarity indicates non-significant differences ($P > 0.05$) between activities among stocks for a sex.

[b] Bold type indicates F_1 activities which are significantly different ($P < 0.01$) from C57BL/6JNctr for both sexes.

Sample Sizes: C57BL/6JNctr ♀ = 51 ♂ = 45. BC10F$_1$ ♀ = 24 ♂ = 28. BC13F$_1$ ♀ = 30 ♂ = 28. BC2F$_1$ ♀ = 21. ♂ = 25. BC6AF$_1$ ♀ = 29 ♂ = 27. BC7F$_1$ ♀ = 23 ♂ = 24

Table III. Relative Deviation in Activity for Those Enzymes of Congenic Lines Where Both Males and Females Were Significantly Different from C57BL/6JNctr

F_1 congenic line	Enzyme	% Difference from C57BL/6JNctr[a]
BC2	Amino acid oxidase	+26%
	Glutamate dehydrogenase	+16%
	Glycerokinase	+16%
	Fructose diphosphate, Aldolase	−14%
BC6A	—	—
BC7	Glutamate dehydrogenase	+14%
BC10	Adenosine triphosphatase	+20%
	Creatininase	+11%
	Glutamate dehydrogenase	+10%
	Malate dehydrogenase	+10%
	Succinate thiokinase	+26%
	Fructose diphosphate, Aldolase	−10%
BC13	Glutamate dehydrogenase	+13%

[a](+) and (−): Indicates percent increase or decrease in an enzymes activity as an average of males and females from an F_1 stock relative to C57BL/6JNctr.

$BC6AF_1$ and $BC13F_1$, while the activities of STK were different in $BC10F_1$ and $BC2F_1$. The enzyme activities of ATPase, CR, GlDH, MDH, STK, and FDP,Ald in $BC10F_1$ were found to be different from those in C57BL/6JNctr mice, and the activities of AAO, GlK, GlDH, and FDP,Ald in the $BC2F_1$ stock were found to be different from those in C57BL/6JNctr mice. The activity of GlDH in C57BL/6NJctr mice was also different from the activities found in both $BC13F_1$ and $BC7F_1$ stocks. No significant differences were observed in across-block comparisons among any stocks. None of the enzymes in the $BC6AF_1$ stock were distinguishable from those in C57BL/6JNctr for both males and females. The $BC10F_1$ carrying two known allelic variants and the $BC2F_1$ carrying three known allelic variants were the only stocks from which multiple enzyme activity changes were observed.

The six enzymes of $BC10F_1$ and the four enzymes of $BC2F_1$ which were different from C57BL/6JNctr represent a total of eight variant enzymes, with two common to both stocks. Several partial correlations were found among these eight enzymes (Table IV). Factor analysis identified three association groups: ATPase, CR, MDH, and STK are one group; AAO, GlK, and GlDH are another; and FDP,Ald seems to be unrelated to either group. Association between enzymes implies that they are causatively dependent or that they are interrelated through some common factor(s). With the exception of FDP,Ald, the enzyme variants of the F_1 stocks were higher than those of C57BL/6JNctr. Glutamate dehydrogenase activity was different from that of C57BL/6JNctr for all the heterozygous stocks except $BC6AF_1$.

Table IV. Partial Correlation Coefficients and Significance Levels

	BC10F$_1$[a]				BC2F$_1$[a]			
	STK	MDH	ATPase	CR	FDP,Ald	GIDH	AAO	GIK
Glycerokinase (GIK)	0.0298	0.1082	0.0983	0.0426	0.1956	0.7502	0.7689	—
	0.5821	0.0450	0.0685	0.4314	0.0003	0.0001	0.0001	
Amino Acid oxidase (AAO)	−0.0239	0.1174	0.0786	−0.0052	0.2388	0.7408	—	
	0.6587	0.0295	0.1459	0.9238	0.0001	0.0001		
Glutamate Dehydrogenase (GIDH)	0.0057	0.0598	0.1041	0.0383	0.1945	—		
	0.9158	0.2688	0.0537	0.4790	0.0003			
Fructose diphosphate aldolase (FDP,Ald)	0.0635	0.1031	0.1404	0.0692	—			
	0.2399	0.0560	0.0091	0.2002				
Creatininase (CR)	0.7287	0.4297	0.6280	—				
	0.0001	0.0001	0.0001					
Adenosine triphosphatase (ATPase)	0.7534	0.6558	—					
	0.0001	0.0001						
Malate dehydrogenase (MDH)	0.4738	—						
	0.0001							
Succinate Thiokinse (STK)	—							

[a]Enzymes selected for analysis were those variant in BC10F$_1$ and BC2F$_1$ stocks, each of which had multiple enzyme differences from C57BL/6JNctr.

DISCUSSION

The mating scheme used in this study provided F_1 stocks which differed from each other and from C57BL/6JNctr only for those portions of the congenic stock genome which might be heterozygous with respect to that of C57BL/6JNctr. Because of the methods by which the congenic stocks were developed, sublines of C57BL/6J mice comparable to the individual congenic stocks but without the marker genes were not available. The use of a single inbred subline, C57BL/6JNctr, in the production of all F_1 stocks minimizes the effects of subline variability for comparisons among F_1 stocks and between F_1 stocks and C57BL/6JNctr mice. However, it does not permit us to unequivocally ascribe observed differences to the specific electrophoretic variant markers. Rather, it allows us to test for the effect of heterogenity resulting from differences in the genome of congenic stocks and C57BL/6JNctr upon enzyme activity levels. In addition to the electrophoretic variant allele(s) of the congenic stock, such differences may also include an allele(s) from the donor strain that was closely linked to, and segregated with, the marker allele during the backcrossing process used to construct the congenic stock; an allele(s) from the donor strain that was not linked to the marker allele but was transmitted to the congenic stock simply by chance; and a variant allele(s) of C57BL/6J which, through genetic drift (Hoi-Sen, 1972; Festing, 1973; Bailey, 1977; Papaionnou and Festing, 1980), has become fixed either in the C57BL/6J sublines used to construct the congenic stocks or in the C57BL/6JNctr used to produce the F_1 stocks.

Enzyme activity comparisons among all stocks have been made and a number of differences observed. The possibility that a portion of these activity differences may be due to random variability cannot be excluded since some differences were small. However, this possibility was minimized by analyzing relatively large populations and through the use of the experimental block design. In addition, the criterion of considering stocks significantly different for an enzyme activity only when both sexes were different further reduced the effects of random variability. In fact, it may have resulted in some true stock differences not being considered significant. Of those stock differences which were considered significant, there was no obvious explanation based upon the variant genes of these stocks for the two enzyme differences observed in comparisons among F_1 stocks. However, there are some interesting speculations which can be made concerning observed differences between the F_1 stocks and C57BL/6JNctr.

The large number of significant enzyme variants found in the $BC10F_1$ and $BC2F_1$ stocks may simply reflect the fact that the backcross markers in those strains carried a larger segment of chromosome from the donor strain to the recipient strain. The BC10 congenic stock contains variants at both *Np-1*

and *Es-10* on chromosome 14 which were linked and are 10 cM apart, with the entire length of this segment believed to be about 30 cM (Womack *et al.*, 1977). The BC2 congenic stock contains variants at *Amy-1, Amy-2* which were linked on chromosome 3, and at the a^+ allele on chromosome 2. Together these can be expected to encompass about 40 cM. There was an increased probability that the larger portion of donor genome of BC2 and BC10F_1 stocks carried additional non-C57BL/6J genes which can exert pleiotropic, heteromeric, or single gene effects upon the realization of measured enzymes.

The marker loci for the BC10 congenic stock were the $ES\text{-}10_c$ (esterase) and $NP\text{-}1_b$ (nucleoside phosphorylase) alleles on chromosome 14. Nucleoside phosphorylase catalyzes the phosphorolytic cleavage of adenosine, guanosine, inosine, and their deoxy analogues (Bergmeyer, 1974). It was therefore directly involved in the availability of ATP, GTP, etc. All of the affected enzymes (ATPase, STK, MDH, GlDH, CR, and FDP,Ald) either use ATP as a cofactor or substrate or were only one step removed from an ATP-requiring reaction in their respective pathways. The alteration at *NP-1* may have a pleiotropic effect upon the realization of the activity of several enzymes which were responsive to the availability of ATP. This speculation was supported by the association among activities of the enzymes ATPase, CR, MDH, and STK as noted by factor analysis. Although GlDH and FDP,Ald were one step removed from an ATP-requiring reaction, their lack of association with the other four enzymes or with each other suggests that additional, independent factors were involved.

The marker loci for the BC2 congenic stock were the *a* locus on chromosome 2 and the amylases on chromosome 3. Amylase was involved in the conversion of glycogen to maltose which, in turn, provides a source of D-glucose for entry into glycolysis. The alterations of $Amy\text{-}1^b$ or $Amy\text{-}2^b$ may account for the variant activity of FDP,Ald, which was a glycolytic enzyme. However, because the activities of AAO, GlDH, and GlK were associated with each other but not with the activity of FDP,Ald, at least two independent factors were required to account for the variant activities of BC2F_1.

The marker locus for the BC7 congenic stock was the mitochondrial MDH, $Mor\text{-}1^b$ allele on chromosome 5. Only the activity of glutamate dehydrogenase differed between this stock and C57BL/6JNctr. No stock difference in MDH activity was observed. It seems improbable that the variant allele at the *Mor-1* locus would be responsible for GlDH activity change since its presence did not elicit clear activity response of its own product. In this case the genetic factor involved in the increase in activity of GlDH was probably one either linked to the *Mor-1*, heterozygous by chance, or the result of genetic drift.

The BC13 marker locus was the *b* allele at esterase 8 on chromosome 7. Again, GlDH activity was found to be different from that in C57BL/6JNctr.

GlDH variants relative to C67BL/6JNctr were found in the BC2, 7, 10, and 13 F_1's. The GlDH activity for $BC6AF_1$ was not significantly different from the C57BL/6JNctr in the females, although it was significant in the males and slightly increased in the females. It was not clear whether all the F_1 stocks carry a common gene variant relative to C57BL/6JNctr and $BC6AF_1$ was just an outlier of this population or whether the other four F_1 stocks each possess separate genetic factors associated with the variant markers which independently impact upon GlDH activity. It was anticipated that there should be more genetic differences resulting from the 20- to 40-cM segments from donor strains than might have occurred through genetic drift. If a gene resides on each variant segment which has a similar effect on GlDH activity, then this enzyme must be responsive to a variety of genetic factors involved in the control of synthesis of amino acids and other related functions in intermediary metabolism. We would consider this situation, where many genetic factors affect a control point enzyme, a heteromeric interaction. Alternatively, if divergence between the two C57BL/6J stocks has occurred such that one of them now possesses a genetic factor which affects GlDH activity, all of the F_1 stocks would be expected to differ from C57BL/6JNctr and exhibit approximately the same activity. The observation that GlDH activity was significantly increased over that of C57BL/6JNctr in males of all five F_1 stocks and females of four F_1 stocks, and even slightly increased in $BC6AF_1$ females, favors the probability that the GlDH differences may be the result of some new gene carried by either the C57BL/6J mice originally used to construct the homozygous congenic stocks or the C57BL/6JNctr mice used in the generation of the F_1 heterozygous stocks. GlDH from all the F_1 stocks and C57BL/6JNctr was analyzed using polyacrylamide gel electrophoresis and isoelectric focusing, and no mobility or isoelectric point differences were noted (data not shown). Although not conclusive, this provides evidence that the genetic factor responsible for the activity differences between C57BL/6JNctr and the four F_1 congenic stocks may not be at the structural gene. Regardless of whether genetic divergence or heteromeric effects of separate, independent genes account for the observed differences, the altered genetic factor responsible must be pleiotropically affecting GlDH activity.

Regulatory genes controlling enzyme synthesis are generally closely linked to their respective structural genes, while postranslational processing genes are typically not linked to their enzymes' structural genes (Paigen, 1979). Since the structural locus of the mapped enzymes having altered activities in the F_1 stocks is either not linked or not known to be linked to the donor portion of the genome, the variations in enzyme activity reported here are not anticipated to be the result of regulatory gene effects, but rather the result of posttranslational processing genes or similar modifying factors. Although the factors responsible for the altered enzyme activities observed in

this study can only be postulated, it is obvious that the activity of a realized enzyme is dependent upon genetic factors other than just the integrity of the structural gene.

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