SELECTION FOR CHOLINESTERASE ACTIVITY IN THE CEREBRAL CORTEX OF THE RAT[1, 2]

THOMAS H. RODERICK[3]

University of California, Berkeley, California
Received March 4, 1960

KRECH, ROSENZWEIG, BENNETT, and KRUECKEL (1954) found strain differences in cerebral cortex cholinesterase activity (ChE) of the rat as well as evidence that some behavioral characteristics might be associated with ChE levels. Since then, studies of ChE and adaptive behavior have afforded several inroads into the understanding of the general biological significance of this enzyme (ROSENZWEIG, KRECH and BENNETT 1958a), beyond that already well established from basic neurological studies.

Selection experiments have been concerned with a wide variety of traits in many different organisms. With respect to biochemical studies, WOODWORTH, LENG and JUGENHEIMER (1952) have described a 50 year selection for protein and oil content in corn. A recent example of the several selection programs, in which the selection criterion is high and low response of an animal to an injected agent, is the experiment of KYLE and CHAPMAN (1953) who selected in the rat for high and low response of the ovary to a standard injected dosage of a gonadotrophic hormone. WEIR and CLARK (1955) successfully selected for high and low blood *p*H in the mouse. No attempt has yet been made to modify by selection the activity of an enzyme.

This selection experiment was undertaken as part of an effort to: (1) find or produce other strains of rats with ChE differences for studies on the relationship of ChE and adaptive behavior, and (2) to obtain information on the genetics of modifiers of enzyme activity.

MATERIALS AND METHODS

Two foundation stocks of rats were used, from each of which a high and a low ChE line have been derived. Both stocks, obtained from animals maintained by the Small Animal Breeding Colony of the Genetics Department, University of California, Berkeley, were genetically heterogeneous, although their genetic backgrounds were different. One stock (RC), obtained from PROFESSOR W. E. CASTLE, was a product of crosses of many different strains from various sources used in his linkage studies. The other stock (RD) was descended from a four-way cross of

[1] Aided in part by grants G-2542 from the National Science Foundation, M-1292 from the National Institute of Mental Health. It also received aid from the United States Atomic Energy Commission and a grant from the Research Committee, Northern Section, Univ. of California.

[2] From a dissertation submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy, University of California, Berkeley.

[3] Present address: Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

four inbred lines of different origin. The four original lines were: (1) U.C. Line A (albino) from Iowa State University, (2) U. C. Line I (agouti hooded) from August Line 990, Wayne University, (3) U. C. Line F (albino) from King A Albino Line, Wistar Institute, and (4) U. C. Line J (albino) from Fischer Line 344, Wayne University.

A double first cousin mating system was used in each generation and in each line throughout the experiment. This mating system was chosen because it maintained maximum heterogeneity for the population size adopted. A more open breeding system would have maintained greater genetic heterogeneity but would have necessitated the care and analysis of a cumbersome number of animals.

Reciprocal crosses were made between two litters within each line in each generation (Figure 1). Matings were made randomly between members of the two litters. After the birth of offspring from these matings, the male parents only were sacrificed for chemical analysis in the 200–250 day age range over which ChE activity remains relatively stable (BENNETT, ROSENZWEIG, KRECH, KARLSSON, DYE, and OHLANDER, 1958). The ChE analyses were made, and the offspring from the highest sire (in the case of a high line or the lowest in the case of a low line) in each of the parental litters were saved, thus affording two new litters with which the process was repeated in the next generation. The measurements on which selection was based were, in all cases, deviations from litter means so that possible maternal and other cage effects did not affect selection.

The assay of ChE activity has been reported elsewhere in detail (KRECH, ROSENZWEIG and BENNETT 1956). The animal was decapitated, and samples from the visual (V) and somesthetic (S) areas of the cerebral cortex were excised (Figure 2). The remainder of the cortex was stripped away, leaving the sub-

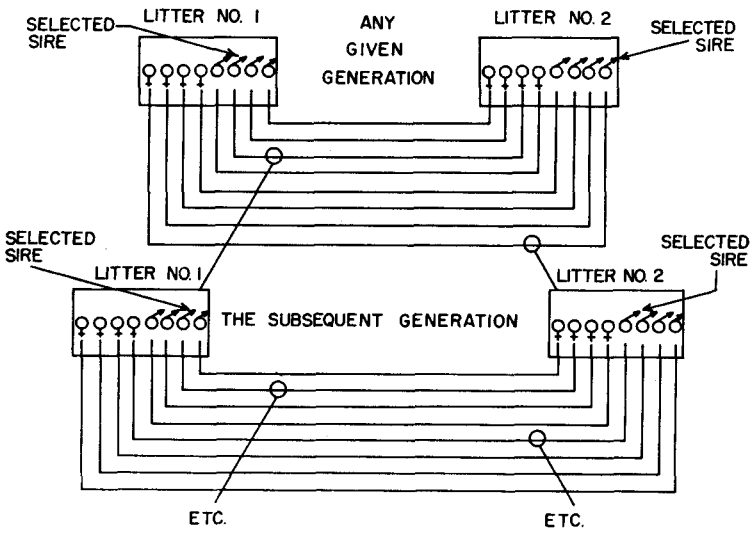

FIGURE 1.—The double first cousin mating scheme.

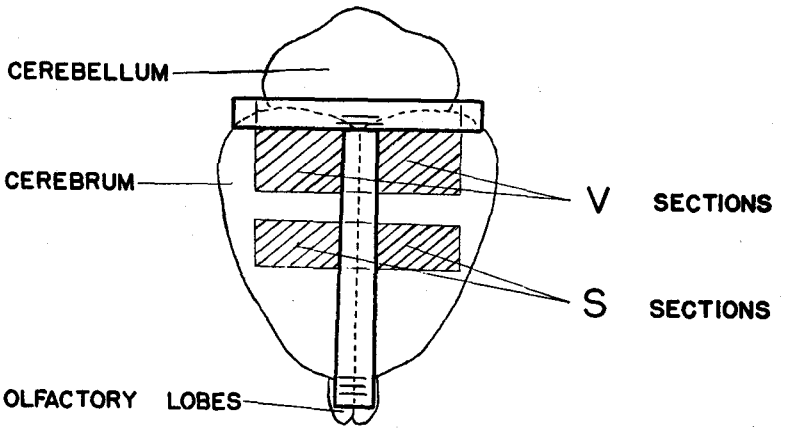

FIGURE 2.—Rat brain and superimposed T square with which the areas to be ablated are delineated.

cortex, olfactory lobes and cerebellum, all of which comprise the subcortical sample. These samples were then weighed rapidly, quick-frozen on dry ice, and stored at —20°C until analysis which followed within the week. Just before the analysis, the sample was homogenized in cold 0.9 percent saline. With the use of a Nielands-Cannon Automatic Titrator (NIELANDS and CANNON 1955), the enzyme activity was determined under standardized conditions by the rate of hydrolysis of acetylcholine perchlorate (ACh). The activity of the sample was expressed as moles of ACh $\times 10^{10}$ hydrolyzed per minute per milligram of tissue. The cortical ChE activity was taken as the average value of the visual and somesthetic areas $[(V + S)/2]$.

RESULTS

The results of selection are given in Table 1 and Figure 3. In Figure 3, each point beyond the foundation stock generally represents the mean of males of two litters taken as a combined array. The standard deviations of the means about each point are also statistics of the male individuals of the two litters grouped together. The environmental effect between generations is large, as indicated by the extent of the vertical zigzagging of the means between generations. Whenever possible, a high line litter and a low line litter were assayed at the same time in an effort to minimize these environmental effects within generations, and therefore to make the high-minus-low value a more efficient statistic. However, it was not usually possible to assay both high litters or both low litters comprising each point at the same time. As a result, some extra day-to-day environmental variation is incorporated into the standard error of each mean. The larger standard errors, from this incorporation, make the high-minus-low values appear less significant than if standard errors depended only on within-litter variation. This alteration is of little importance, as the litter means within each line in each generation vary so little; the within generation variance is obviously negligible compared with the between generation variance

TABLE 1

Results of selection for ChE activity.
Number (N), *mean* ($\bar{x}$), *standard deviation* (s), *standard error* ($s_{\bar{x}}$)

Sel. gen.*	Line	N	$\bar{x}$	s	$s_{\bar{x}}$	High minus low	Line	N	$\bar{x}$	s	$s_{\bar{x}}$	High minus low
S_0	RD	14	59.04	4.53	1.21		RC	14	57.76	6.42	1.05	
S_1	RD high	8	59.65	3.51	1.24	— 1.77	RC high	4	57.65	2.23	1.11	5.56
	RD low	7	61.42	5.41	2.05		RC low	9	52.09	5.38	1.79	
S_2	RD high	9	62.30	3.28	1.09	14.31	RC high	8	60.41	5.63	1.99	3.99
	RD low	8	47.99	3.03	1.07		RC low	6	56.42	5.55	2.27	
S_3	RD high	10	58.77	4.30	1.36	5.52	RC high	9	53.29	2.62	0.87	7.91
	RD low	7	53.25	2.44	0.92		RC low	6	45.38	3.91	1.59	
S_4	RD high	10	63.60	3.45	1.09	12.48	RC high	9	58.81	3.32	1.10	6.95
	RD low	8	51.12	2.27	0.80		RC low	8	51.86	2.71	0.96	
S_5	RD high	11	61.03	4.09	1.23	13.91	RC high	15	54.27	3.31	0.86	9.13
	RD low	8	47.12	2.19	0.77		RC low	13	45.14	2.43	0.68	
S_6	RD high	8	60.67	2.11	0.75	12.04	RC high	5	64.37	2.43	1.09	16.54
	RD low	8	48.63	2.67	0.95		RC low	8	47.83	2.87	1.01	

* Selected generation.

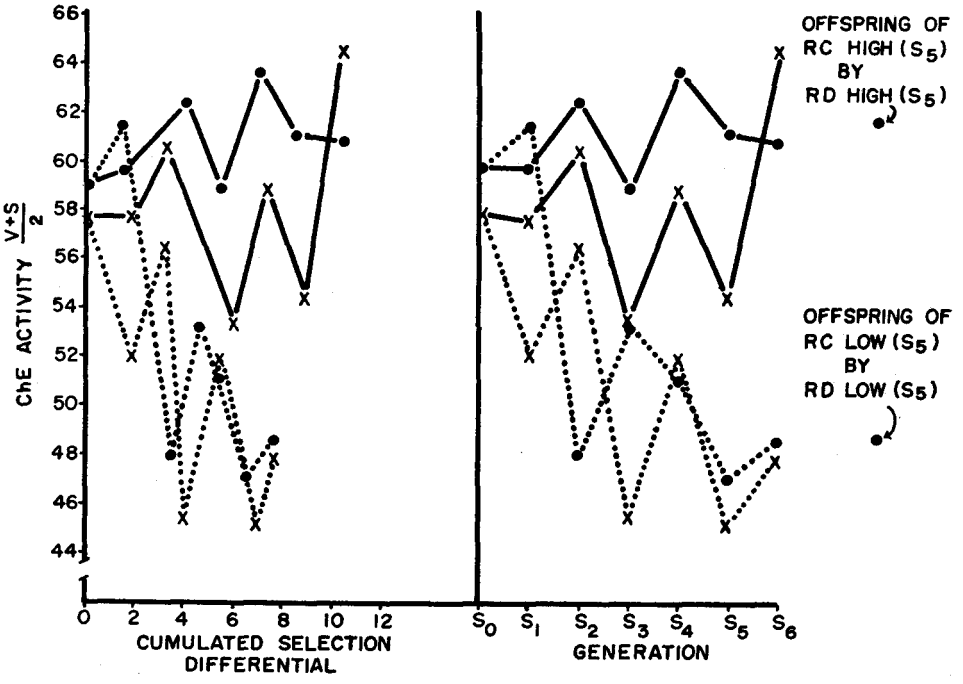

FIGURE 3.—Results of selection for cortical ChE.

as indicated by the values of $s_{\bar{x}}$ compared with the generation shifts within a line.

Selection was successful almost immediately, and the lines remained apart in the succeeding generations. In all the 12 pairs of generation points represented in both stocks, only one pair, RD S_1, is reversed from the direction selected. The probability, that of 12 independent comparisons, 11 or more would by chance be in agreement with the direction of selection, is

$$(\tfrac{1}{2})^{12} + 12(\tfrac{1}{2})^{11}(\tfrac{1}{2}) \text{ or } 13(\tfrac{1}{2})^{12} = 0.0032.$$

There were no overlappings of the high and low distributions for generations five and six for the RC lines and generations two, four, five and six for the RD lines.

To ascertain whether selection continued to separate the high and low lines as the experiment progressed, regression lines of the high-low difference for both stocks were estimated, each generation contributing one point to the array to which the lines were fitted (Figure 4). Also, a regression line was fitted to an array of points based on the average of the divergence of both stocks for each generation. An analysis of variance was performed to test the significance of these regression lines.

$P = 0.18$ (regression line of separation of RD lines).
$P = 0.02$ (regression line of separation of RC lines).
$P < 0.01$ (regression line of average of separation of RD and RC lines).

From these data it is observed that selection was successful in separating the lines by approximately two ChE units per generation. The direction and extent of the deviations from regression in the latter generations do not give any indication of an incipient plateau.

The significant positive linear regression of the separation of lines is evidence that selection acted upon a polygenic system. Crosses were made between the S_5 generations of the two stocks so as to form a high-by-high line and a low-by-low line. The mean ChE values of the offspring of each cross are comparable in magnitude to the means of the parental stocks for the S_6 generation (Figure 3). These results indicate that even stocks of very different ancestry do not differ with respect to genes showing dominance for ChE activity, or if they do, that the direction of dominance at different loci is almost exactly balanced in plus and minus directions in both high or both low lines. The latter alternative would be exceedingly unlikely. Epistatic effects of gene differences between the stocks, if present, would be expected to result in shifts of the ChE values of the progeny. If different epistatic effects existed between the two stocks, the means of the offspring between the stocks would be expected to shift from their midparent values. Unless identical genes were selected in the two unrelated foundation stocks, it can be concluded that the high and low lines differ mainly in genes that are largely additive.

Heritability: The gain over reach, or realized heritability (h^2), was calculated as follows: The value of the reach for each selected sire was taken as his deviation

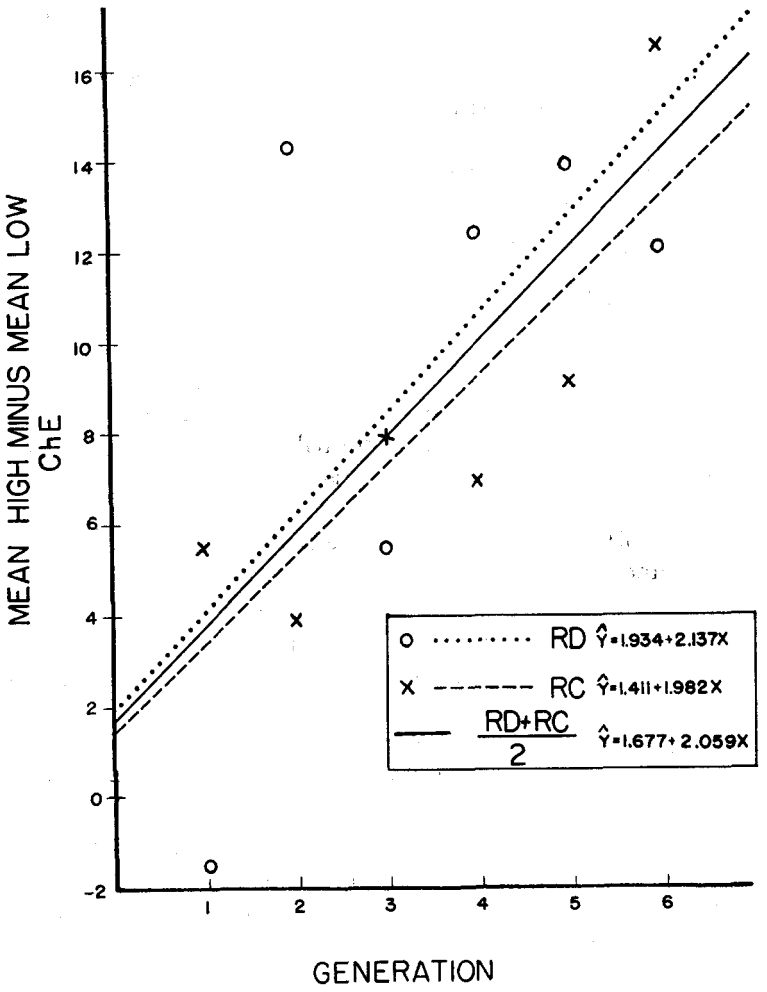

FIGURE 4.—The regression of mean high ChE minus mean low ChE on generation.

from his own litter mean. These values were averaged for the selected sires of the two litters of each line within each generation and then divided by two, because only sires were selected. The gain was estimated as the average value of the unweighted litter means of the succeeding generation, minus the average value of the sires' unweighted litter means. The h^2 estimates (Table 2), therefore, are within-litter estimates where the gain is approximately some constant times the additive genetic variance within litters. The reach is the same constant times the total variance within litters and would not include any variance from maternal or other litter effects.

Although the separations of high and low selected lines give adequate estimates of the average reaction of each strain to plus and minus selection, the reason for the apparent extreme response in both low lines remains an enigma.

TABLE 2

Realized heritability (sum gains/sum reaches)

Stock	High	Low	High and low combined	All lines combined
RD	0.13	1.39	0.67	
				0.78
RC	0.58	1.31	0.89	
Estimates when gain is based on regression line				
RD			0.80	
				0.78
RC			0.74	

It is possible that the whole assay technique has for some reason gradually yielded lower ChE values, although assays of other strains, for which the mean values of ChE are well established, have shown no downward trends. Inbreeding depression is not a factor because the high-by-high and low-by-low crosses, with inbreeding coefficients of zero, fall right in place with the S_6 generation of the original selected lines.

The numerators of h^2 computed by gain over reach depend only on the means of the litters of the first and final generations. To reduce this possible sampling error and to give equal weight to the gains in intermediate generations, total gain was also computed from the least squares regression line of Figure 4. It is, to some extent, coincidental that both estimates for all lines combined are exactly the same. It is interesting to note that the selection differentials were almost identical between the two high lines and between the two low lines. Wherever possible the extreme animals were selected, and no attempt was made to control the differentials in any way.

At the outset of the program, heritabilities were estimated in two other ways. An estimate of 0.163 with 95 percent confidence limits of 0.004 and 0.692 was made from a comparison of five inbred strains. This estimate is based on the assumptions that the strains are completely homozygous, and that they are unrelated strains picked at random from a hypothetical population of strains. Since these assumptions are not completely valid, the estimate is probably an underestimate of h^2. The other estimate was obtained by comparing phenotypic and genotypic relationships between sibs of the foundation stocks. The RD h^2 estiestimate was 0.069 with 95 percent confidence limits of —.570 and 1.559. The RC h^2 estimate was 1.423 with 95 percent confidence limits of 0.525 and 1.947. These heritabilities are estimated assuming that the genetic variance between sibs is half that of the whole population. If this assumption is not completely true (and there is doubt about it in the RD stock, as it was derived from a cross between two lines descended from an original four-way cross), then the estimate would tend to be biased in the minus direction. Also, the assumption is made that besides common genetic effects, no common litter effects, such as maternal effects, prevail; such effects would produce a bias in the plus direction.

Natural selection: That success in the high direction was markedly less than

in the low direction suggests that natural selection may have opposed upward selection. The high lines were in one respect characteristic of animals with lowered fitness if body weight in this case can be considered a manifestation of fitness. Figure 5 summarizes the values of the mean body weights at the time of ChE determination for each line for the S_5 and S_6 generations plus the S_1 generation of the high-by-high and low-by-low crosses. Earlier generations were not weighed. Again, as in the ChE summary, the two or three litters making up each point are taken as single arrays in computing their standard errors. Visual comparison of the data of the high and low lines both between and within stocks, and between the crosses reveals an obvious negative association between litter mean cortical

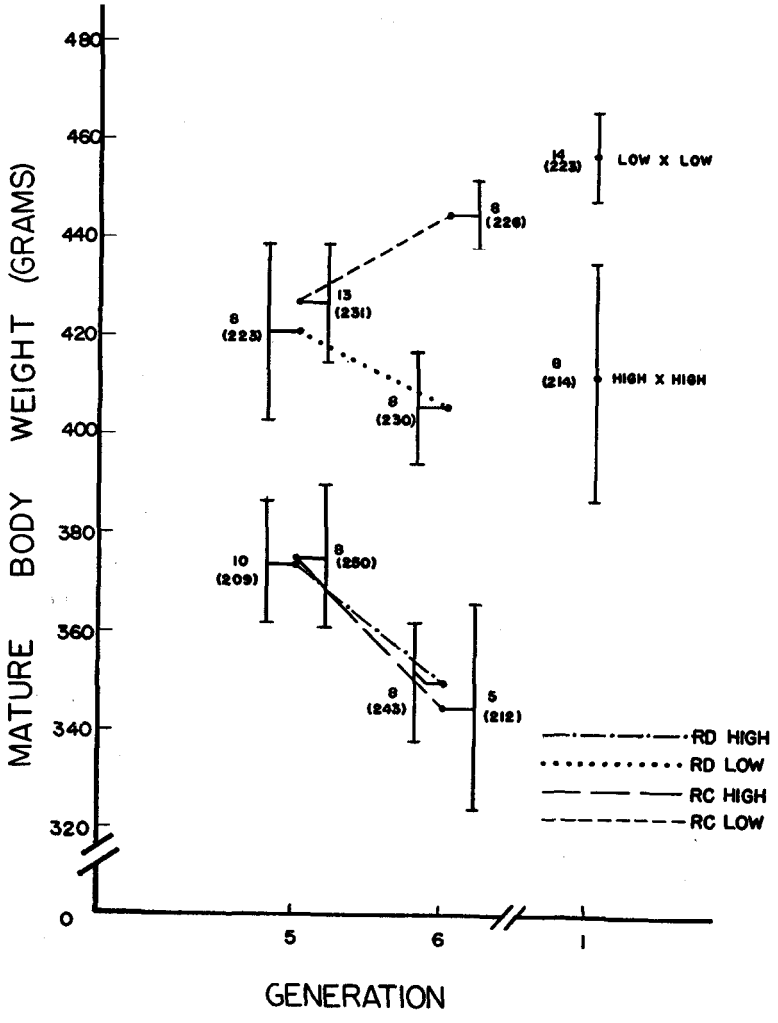

FIGURE 5.—Mature body weight with N, $s_{\bar{x}}$, and average age in parentheses.

ChE and litter mean body weight. Although heterosis appears to be manifested in the body weight means of both cross lines, the separation remains.

Disregarding the means and taking only *within-litter* correlations between ChE and body weight (i.e., a value of each variate is the deviation from the litter mean) of all animals of generations S_5 and S_6 and the S_1 of the cross lines, led to the following result: within-litter correlation of ChE and body weight $= -0.19$; $N = 97$; $P < .05$ (two-tail test). The negative relationship thus holds both between the lines and within the litters. That the within-litter correlation is in part due to a genetic correlation is evidenced by the separation of the body weight means through selection for ChE.

There are now some preliminary behavioral data on the selected lines (KRECH, personal communication) which seem to indicate that the high ChE lines are poorer than the low lines in adaptive behavior as measured by a variety of maze situations. Adaptive behavior may be considered a manifestation of fitness.

Reproductive fitness in terms of percent sterility did not differ between the high and low lines. Also mean litter size was apparently not affected by selection.

Information from variables concomitant to selection concerning the mechanism of selection: It is important to understand the physiological mechanisms through which selection has acted to alter the phenotypic expression of ChE activity if the implications of enzyme activity alteration by genetic means are to be considered. In these experiments, because activity is measured per unit weight of tissue, it is necessary to know, for example, if there are merely more cells per unit weight in high ChE animals than in low ChE animals (with the assumption that more cells mean more nucleoprotein and therefore more enzyme ChE). For the psychologist interested in ChE activity and behavior, important generalizations from psychological studies on these lines might be based on the hypothesis that the mode of action of selection has been primarily through some direct biochemical alteration of ChE activity. If ChE activity had been altered because of gross neurological tissue changes, then it would be more reasonable to study gross tissue changes and behavior, with ChE activity as an interesting side light. It is desirable to distinguish between such mechanisms as this and the more direct mechanisms probably of an intracellular biochemical nature.

During the selection program other variables than ChE activity were measured to find out if any correlated responses to selection did occur. From these observations an attempt has been made to begin the clarification of the mode or modes of the physiological action of selection.

Brain size: With the use of vernier calipers, lengths and widths of the cerebrum were measured for all assayed animals beginning with generation S_2 (Figure 6). The probability that chance alone could have permitted the consistant difference observed in lengths for all generations measured is:

$$2\,[(\tfrac{1}{2})^{11} + 11(\tfrac{1}{2})^{10}(\tfrac{1}{2})] = 0.017 \text{ (two-tail test)}$$

for widths:

$$2\,[(\tfrac{1}{2})^{11} + 11(\tfrac{1}{2})^{10}(\tfrac{1}{2}) + 55(\tfrac{1}{2})^{9}(\tfrac{1}{2})^{2}] = 0.065.$$

The length and width differences, which are in the same direction, when con-

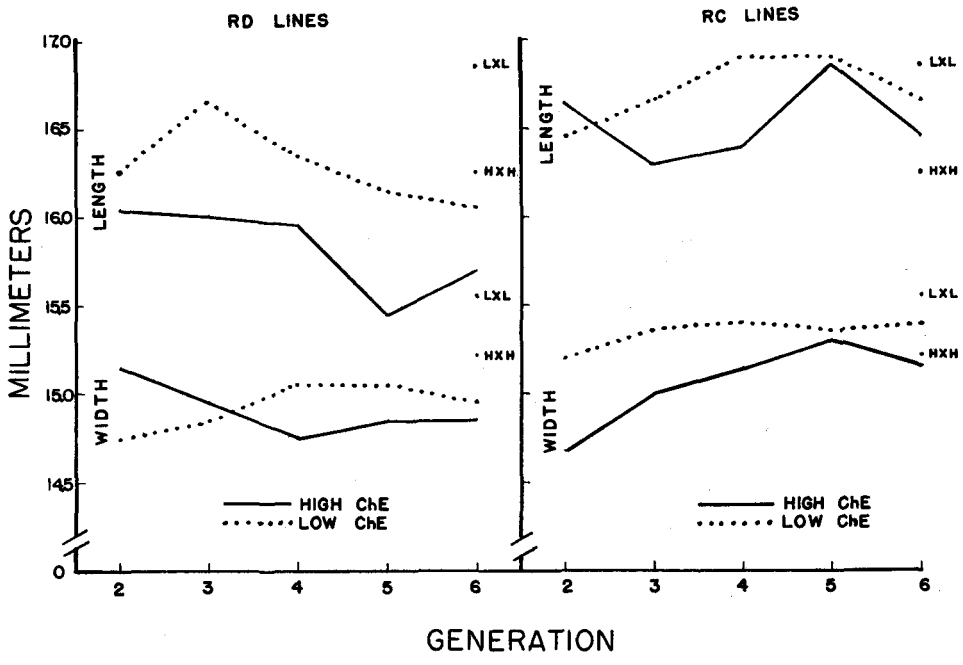

FIGURE 6.—The change in cerebrum length and width with selection for ChE.

sidered together indicate that the smaller brain dimensions of the high ChE lines is highly significant.

ROSENZWEIG, KRECH and BENNETT (1958a) found a gradient of ChE activity over the cerebral cortex of the rat; ChE decreases from anterior to posterior. Thus, the somesthetic area of the cortex has a higher activity than the visual area. The *V* and *S* areas of the cortex (before they were ablated for assay) were carefully delineated on the exposed brain by the use of a small plastic T square (Figure 2). The T square was centered on the cortex and the position of the *S* section was determined by delineating a specific area at the center of the cortex. The position of the *V* section was then determined by measuring a definite distance posterior to the *S* section. Because the *S* areas were taken from the center of the cortex, it is reasonable to consider them physiologically comparable between the high and low lines if selection has not affected the gradient in some fashion. The *V* sections might, however, for reasons of differences in brain length, be histologically different between high and low animals, since the *V* sections were determined by marking a fixed distance from the *S* section. However, since the ChE values of the *V* sections remained approximately 6.3 ChE units below the *S* sections in both high and low lines throughout the program, there is no evidence that possible sampling of histologically different areas is either assisting or counteracting ChE separation between high and low lines.

Brain specific gravities: Since brain dimensions were smaller in the high lines, it was thought possible that brain densities might also have changed with selec-

tion and that a study of any differences in brain density might shed further light on the physical basis for ChE separation brought about by selection. Brain specific gravities were measured from animals of the S_6 and S_7 generations of the four original lines plus the S_1 of the cross lines (Table 3). Since immersion of the brains in water for the determination would have altered the weights of the cortex in any subsequent ChE determination of the same brains, the regular ChE assayed males could not be used. Instead, females sibs of the males were used. The females were sacrificed between 200 and 300 days of age, their brains removed and weighed in air, and then weighed in distilled water. The brain weight in air divided by the loss of weight in water (corrected for temperature variation of the water) was taken as the specific gravity of the brain. A test of the difference between the means of the high and low line shows that the animals from the high ChE lines have a significantly greater brain density than do animals from the low ChE lines:

$$F^{1}_{36} = \frac{6917.0}{852.2} = 8.12,$$
$$P < 0.01.$$

The interaction and between stock variations were not significant.

This association, of course, does not help to explain the ChE separation from selection, as ChE is measured per unit weight tissue; however, the high lines would show even higher ChE if it were determined per unit volume instead of weight.

Since protein is denser than water and most other major cell constituents, an important question is whether the protein percentage has merely increased in the brain cells of the high line animals and decreased in the low, to give the observed ChE separation. An investigation of protein percentage by weight in the cortical and subcortical brain tissues has been undertaken (E. L. BENNETT, personal communication), and the preliminary results do not suggest any association with ChE.

Subcortical ChE: On some of the animals from the S_5 and S_6 of the original and the S_1 of the cross lines, subcortical ChE values were determined. Subcortex here means the whole brain minus the cerebral cortex which was stripped off. The separation of subcortical ChE between the lines was highly significant (Table 4). The within-litter coefficients of variation for cortical ChE and subcortical ChE were 4.79 percent and 3.26 percent, respectively.

TABLE 3

Means of brain specific gravity

Stock	Selected high ChE lines		Selected low ChE lines	
	$\bar{x}$	N	$\bar{x}$	N
RD	1.0405	7	1.0390	7
RC	1.0396	7	1.0350	7
Cross lines	1.0395	7	1.0378	7

TABLE 4

A comparison of cortical ChE and subcortical ChE of the same animals with respect to their separations between high and low lines selected for cortical ChE

		Cortex					
Stock	Generation	High $\bar{x}_1$	Low $\bar{x}_2$	$\bar{x}_1-\bar{x}_2$	t	df	P
RD	S_5	57.11	44.96	12.15	12.15	9	<.0005
	S_6	60.68	48.63	12.05	20.00	14	<.0005
RC	S_5	53.40	44.59	8.81	11.17	15	<.0005
	S_6	64.37	47.83	16.54	21.94	11	<.0005
Cross lines	S_1	61.46	48.48	12.98	18.88	16	<.0005

		Subcortex					
Stock	Generation	High $\bar{x}_1$	Low $\bar{x}_2$	$\bar{x}_1-\bar{x}_2$	t	df	P
RD	S_5	128.30	122.50	5.80	3.09	9	<.01
	S_6	134.25	125.88	8.37	6.27	14	<.0005
RC	S_5	130.88	130.33	0.55	0.19	15	>.25
	S_6	152.20	122.75	29.45	9.67	11	<.0005
Cross lines	S_1	132.75	125.79	6.96	6.85	16	<.0005

TABLE 5

Summary of correlations (r) *by lines between cortical and subcortical ChE*

Line	*r*	*N*
RD high	+ .96	11
RD low	+ .48	16
RD lines combined	+ .56	17
RC high	+ .73	13
RC low	— .04	17
RC lines combined	+ .50	30
High-by-high	+ .71	4
Low-by low	+ .37	14

The correlation between cortical and subcortical values was estimated in a number of ways:

	r	*N*
over-all	+.68;	75
within litters	+.50;	75
between litter means	+.77;	20
between line means, generations separate	+.82;	6

All are significant at the one percent level, although none are independent. These correlations are broken up into correlations within individual lines in Table 5.

Although there do not appear to be any differences in the magnitude of these correlations between the stocks, there is some indication that the high selected lines have a higher correlation. (The insignificantly negative RC low correlation may have resulted from sampling error. Although the difference between the RC high correlation and the RC low correlation is significant at the one percent level, more data on the RC animals would be helpful to determine whether the magnitude of the difference is as great as observed in these data.) However, ROSENZWEIG, KRECH, and BENNETT (1958b) found lower correlations in higher ChE lines in two comparisons, one of which involved other animals from the RC stock. The differences observed here may be fortuitous.

The conclusion to be drawn from these data is that a considerable positive correlation exists between cortical and subcortical ChE. The author feels that the same general conclusion can be drawn from the data of ROSENZWEIG, KRECH, and BENNETT (1958b). The fact that their correlations are not within-litter correlations may account for the general difference in magnitude between their correlations and those reported here.

Thus, the estimate of the correlation between lines is similar to the correlation estimate within litters. Their similarity in magnitude suggests they are estimates of the same value and is evidence for a large genetic component of the correlation.

An important consideration is whether cortical ChE as measured by $(V + S)/2$ is as highly correlated with subcortical ChE as it is with ChE of other areas of the cortex. The motor sections (sections of the cortex comparable to the S section in size but more anterior in location) of 16 animals of the cross lines were assayed. These animals comprised two high and two low line litters of which the mean values were:

Line	Litter	Litter mean ChE (M section)
H × H	514a	70.0
	515	71.8
L × L	618	58.7
	615a	58.5

The within-litter correlation between $(V + S)/2$ and M of all 16 animals was .74 which is significant at the one percent level. The similarity of magnitude of the correlations suggests that selection for ChE in an area of the cortex will affect the ChE in the unselected subcortex almost as greatly as ChE in any other unselected area of the cortex.

Brain weight: Although the brain densities are greater in the high lines, the brain weights are lower. Figure 7 shows the means of the brain weight for the S_5 and S_6 generations of the original lines and the S_1 of the cross lines. The negative association between these two variables holds in general, but is more obvious between the lines within each stock and between the cross lines. If body weight and brain weight were correlated, it would not be surprising to find the ChE-brain weight association since, as already discussed, a negative correlation exists between body weight and ChE. Comparing the mean body and mean

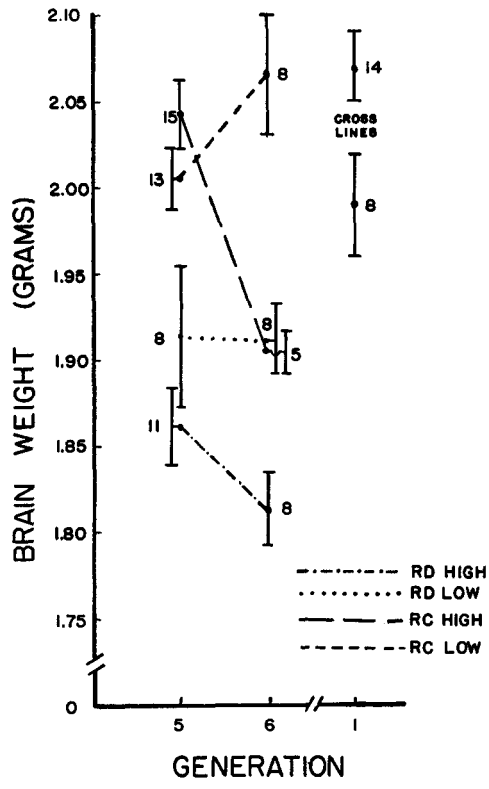

FIGURE 7.—Mean brain weights of each line with N and $S_{\bar{x}}$.

brain weights of these animals, a correlation of +.83 ($P < .01$) was found.

Correlations between these three variables were also estimated within the litters, i.e., taking the value of each variate of each animal as its deviation from its own litter mean. The results based on 97 animals are summarized here with the one-tail probability of each correlation.

ChE, brain weight	$r = -.22$; $P = .010$
ChE, body weight	$r = -.19$; $P = .025$
body weight, brain weight	$r = +.33$; $P < .001$

These three correlations were evaluated for each of the two stocks and the cross lines, respectively (Table 6). Also, from 75 of the 97 animals used in estimating these correlations, data of the subcortical ChE were obtained as already presented. In Table 6 are also the correlations between subcortical ChE and brain weight, and subcortical ChE and body weight. The within-stock correlations between the ChE variables and the weight variables in general support in direction the total correlations. KRECH, ROSENZWEIG and BENNETT (1958) found a strain difference in the magnitude of the correlation between subcortical ChE and brain weight. More data on the individual stocks would be necessary to determine if

TABLE 6

Within-litter correlations and partial correlations for the variables: cortical ChE, subcortical ChE, brain weight, and body weight where w *is subcortical ChE,* x *is cortical ChE,* y *is brain weight,* z *is body weight*

	RD stock	RC stock	Cross lines	Total
N	27	30	18	75
r_{wy}	− .64***	− .32*	− .20	− .40***
r_{wz}	− .44**	− .26	− .12	− .31***
r_{yz}	+ .54***	+ .16	+ .52**	+ .38***
$r_{wy.z}$	− .53***	− .29*	− .16	− .33***
$r_{wz.y}$	− .15	− .22	− .02	− .18
N	34	41	22	97
r_{xy}	− .26	− .17	− .26	− .22*
r_{xz}	− .13	− .25*	− .19	− .19*
r_{yz}	+ .56***	+ .01	+ .61***	+ .33***
$r_{xy.z}$	− .23	− .17	− .19	− .17*
$r_{xz.y}$	+ .02	− .26*	− .04	− .13

* $P < .05$.
** $P < .01$.
*** $P < .005$.

any strain differences exist with respect to the magnitude of these correlations. In addition, it may be noted from these data that the correlations of the ChE variables are generally greater with the brain weight than with body weight. That the negative association does not entirely disappear between the ChE variables and body weight when brain weight is held constant (five out of the six partial correlations $r_{xz.y}$ are negative, and one is significant by itself) suggests that the negative association between the ChE variables and brain weight is probably not the only influence in the ChE-body weight correlation. Also from these data, as was found by KRECH, ROSENZWEIG and BENNETT (1958), of the two ChE variables, the subcortical ChE has the greater negative correlations with the weight variables. This greater negative association may be real, or it may be that more of the subcortical ChE variation is genetic than the cortical ChE; the lighter weight of the cortical ChE brain samples may subject those samples to more experimental weighing error.

That the total within-litter correlations support the between line associations observed is evidence for the likely possibility that selection for ChE, which was within-litter selection, has given rise to the association of these variables between the lines. It is difficult to draw any conclusions about the mode of action of selection from these data other than the tentative suggestion already made—i.e., if body weight, and hence brain weight, are manifestations of fitness, then higher ChE may produce less fit and hence less heavy animals. The dimensional changes that occur (with the possibility that brain density changes result from them alone) may be prerequisites to brain weight changes, which in turn may have to occur for some unknown reason in order to make ChE separation from selection possible. Until more exact studies are performed, which might include

histological and cytological work, the basis for these associations remains conjecture.

A positive, but insignificant, correlation between brain weight and cortical ChE was found by KRECH, ROSENZWEIG and BENNETT (1958), who also found a significant negative correlation between brain weight and subcortical ChE only. Since from their findings, and those presented here, subcortical ChE and brain weight are negatively correlated, it is not surprising that cortical ChE and brain weight are also negatively correlated as the cortical and subcortical ChE activities are themselves highly positively correlated.

Body color and color patterns: Since obvious single-gene differences were present in the stocks, a study of any possible association between them and ChE was made. The following alleles were present:

RD stock	RC stock
agouti, nonagouti	agouti, nonagouti
hooded, nonhooded	hooded, nonhooded
albino, nonalbino	albino, nonalbino
	dilution, nondilution
	shaker, nonshaker
	hairless, nonhairless

Although some tendency for fixation of alleles occurred, there was no pattern of association between direction of selection and the increase in frequency of a particular allele.

CONCLUSIONS

The correlated responses to selection, which were found in particular traits and not in others, help to elucidate the physical basis of the action of selection to produce the ChE separation. The positive association of brain density with ChE suggests that further attempts to determine percentages by weight of cell and tissue constituents which differ in density may be helpful to clarification of this physical basis. For example, the less dense, and as yet unstudied, myelin of the brain may have increased in the low lines to lower the ChE activity as it is measured. The lack of correlated response of percent protein by weight rules out the simple explanation that selection has merely altered protein percentages causing the ChE changes and brain density changes.

The negative association of brain and body weight with cortical and subcortical ChE is not easily explained. As has been already mentioned, if brain weight and body weight as well as adaptive behavior are manifestations of fitness, then the high lines may be less fit than the low lines. Corroboration for this hypothesis comes from the lesser response to selection in the high lines. Perhaps an optimum ChE level already existed in the foundation populations, and any value above that level is detrimental to the animal. However, the high lines do not appear less fit in other attributes of fitness such as sterility and number of offspring. Also, since h^2 in the low direction exceeds 1.0 in both stocks, it may be that all the ChE values of the later generations should actually be higher than observed for some reason.

The high correlated response of subcortial ChE indicates that the genes affecting the variation of ChE of the cortex are to a great extent the same genes which affect the variation of subcortical ChE. Therefore, selection has not been specific to the cortex. It would be of interest to note if ChE levels of other tissues, such as the blood, have also been altered to any extent.

It may be said that selection for cortical ChE, possibly through some cell or tissue constituent which could be characterized in one way by its density, has produced changes in ChE activity in the whole brain.

SUMMARY

Selection for high and low cortical cholinesterase activity (ChE) in the rat has been successful in two genetically heterogeneous populations of different ancestry. The ChE separation of the lines was continuous over the six generations, implying that the genetic variation of ChE was determined by several genes. The greater part of the within-litter variation of ChE was due to genetic variation (realized $h^2 = 0.78$). Some animals of each line of the S_5 generation were mated to produce two cross lines between the stocks—a high-by-high line and a low-by-low line. The ChE values of the cross lines were comparable to their respective parental lines of the S_6 generation.

Selection in the high direction was much less successful than in the low direction. Natural selection possibly may have countered artificial selection, but because of other data (h^2 in the low direction exceeded 1.0 in both stocks) it appears that other undetermined factors must have been operating.

Other traits were studied for correlated response to selection. The significant associations found were: (1) brain size negatively associated with ChE; (2) brain density positively associated with ChE; (3) brain weight correlated with body weight, both of which were negatively associated with ChE and subcortical ChE; and (4) subcortical ChE positively associated with ChE. The studies of correlated responses indicate that the successful selection for cortical ChE, possibly through some cell or tissue constituent which could be characterized in one way by its density, has produced parallel changes in ChE activity in the whole brain.

ACKNOWLEDGMENT

I am particularly indebted to PROFESSOR EVERETT R. DEMPSTER for his guidance and counsel throughout the course of the investigation. I am grateful for the opportunity to have associated in this program with PROFESSOR DAVID KRECH and DR. EDWARD L. BENNETT and for the fruitful discussions with them. I also wish to express my appreciation to MRS. HILDA KARLSSON RODERICK who performed all the brain surgery and ablations for the project.

LITERATURE CITED

BENNETT, E. L., M. R. ROSENZWEIG, D. KRECH, H. KARLSSON, N. DYE, and A. OHLANDER, 1958 Individual, strain and age differences in cholinesterase activity of the rat brain. J. Neurochem. **3**: 144–152.

KRECH, D., M. R. ROSENZWEIG, E. L. BENNETT, and B. KRUECKEL, 1954 Enzyme concentrations in the brain and adjustive behavior patterns. Science **120**: 994–996.

KRECH, D., M. R. ROSENZWEIG, and E. L. BENNETT, 1956 Dimensions of discrimination and level of ChE activity in the cerebral cortex of the rat. J. Comp. and Physiol. Psychol. **49**: 261–268.

1958 Correlation between brain cholinesterase and brain weight within two strains of rats. Am. J. Physiol. **196**: 31–32.

KYLE, W. K., and A. B. CHAPMAN, 1953 Experimental check of the effectiveness of selection for a quantitative character. Genetics **38**: 421–443.

NIELANDS, J. B., and M. D. CANNON, 1955 Automatic recording *p*H instrumentation. Anal. Chem. **27**: 29–33.

ROSENZWEIG, M. R., D. KRECH, and E. L. BENNETT, 1958a Brain chemistry and adaptive behavior. *Biological and Biochemical Bases of Behavior*. Univ. of Wisconsin Press. Madison, Wisconsin.

1958b Brain chemistry and adaptive behavior. *Ciba Found. Symp. on Neurological Basis of Behavior*. J. and A. Churchill Ltd. London, England.

WEIR, J. A., and R. D. CLARK, 1955 Production of high and low blood *p*H lines of mice by selection and inbreeding. J. Heredity **46**: 125–132.

WOODWORTH, C. M., E. R. LENG, and R. W. JUGENHEIMER, 1952 Fifty generations of selection for protein and oil in corn. Agron. J. **44**: 60–65.