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INDIVIDUAL, STRAIN, AND AGE DIFFERENCES IN
CHOLINESTERASE ACTIVITY OF THE RAT BRAIN¹

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We have previously published data (ROSENZWEIG, KRECH and BENNETT, 1958) which indicate the existence of a relationship between the level of cholinesterase (ChE) activity in the rat's cortex and his adaptive behaviour. Since our interest was in the normal enzymic control of behaviour, experimental interference with the animal's enzymic activity has been avoided in most of our work. Such studies, correlational in nature, depend upon accurate measurement of normal individual differences in the behaviour of the animal and of normal individual differences in enzymic activity.

Early in our work it became apparent that two important variables which conditioned the relationship between ChE and adaptive behaviour were the age and the genetic background of the animal. In the course of the investigations, therefore, considerable data have been accumulated on individual differences in brain ChE activity and in brain weight in the rat as a function of age and strain.

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2. Radiation Laboratory.

3. Department of Psychology.

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There are a number of studies in the literature which present growth curves for ChE activity in the brain of the rat (NACHMANSOHN, 1939; METZLER and HUMM, 1951; ELKES and TODRICK, 1955; COHN and RICHTER, 1956). These studies, however, suffer from several defects in terms of our interests. In most studies the investigator's primary concern was with the first month of life after birth, and the growth curves after that period are usually based upon an exceedingly small number of cases or upon extrapolations. In some studies the number of animals assayed at every age period is so small as to make it worthless to seek any estimate of individual variability. Indeed, in some studies the results of "two or three animals" are pooled and only the means are reported. In other studies standard deviations as well as the means are given, but these standard deviations are based upon successive measures of the same pooled tissue sample. Thus these standard deviations indicate reliability of the experimenter's measurement techniques, but do not give any information on variability among the experimental animals. Another common attribute of these studies is the failure to report the strain of rat used. Where the strain is specified, no comparable data for another strain are given and so it is impossible to determine the role of genetic factors in brain enzyme activity.

Quite aside from our own concern with individual differences in brain ChE activity--a concern necessitated by our specific experimental problem--there has been a growing interest in individual differences and variability of biochemical factors in organisms (WILLIAMS, 1956). Because data on individual differences in enzymic activity in the mammalian brain, and ChE activity in rat brain specifically, have rarely been reported in the literature, our data may be of general value.

MATERIALS AND METHODS

The data reported in this paper have been obtained from male rats of two of the strains regularly maintained in the Department of Psychology colony: S₁, descendants of the Tryon Maze-Bright strain (TRYON, 1929) and S₃, descendants of the Tryon Maze-Dull strain. It should be emphasized that these animals are "descendants," since Tryon's selective breeding program of rapid and slow maze learners ended many years ago, and subsequent genetic drift as well as accidental cross-breeding may have changed these strains considerably from their original status. Some of the rats whose data are reported were given behavioural tests prior to sacrifice, others received no such testing. In this paper only the biochemical and brain weight data are presented.

When an animal was ready for chemical analysis, it was decapitated, and 40 to 50 mg. of tissue from the visual and 25 to 35 mg. from the somesthetic areas of the cerebral cortex were removed by gross dissection. The samples removed and the procedure used are described in detail elsewhere (KRECH, ROSENZWEIG and BENNETT, 1956; ROSENZWEIG, KRECH and BENNETT, 1958). The rest of the dorsal surface of the cortex was then stripped off, and the remaining brain, including the olfactory bulbs and the cerebellum was kept as a third sample. The three samples as well as the stripped off portion of the cortex were weighed rapidly on a projection analytical balance. Originally the samples were analyzed the day they were removed, but more recently we have been freezing the samples rapidly on blocks of dry ice and storing them at -20°C. The elapsed time from decapitation of an animal to placing the samples on dry ice does not exceed 10 minutes. Storage of samples for periods up to two weeks does not appear to affect ChE activity.

An homogenate of the tissue sample was prepared in cold 0.9 per cent

NaCl, and the enzymic activity was determined under standardized conditions by the rate of hydrolysis of acetylcholine perchlorate (ACh). The procedure of analysis has been reported previously (KRECH, ROSENZWEIG and BENNETT, 1956; ROSENZWEIG, KRECH and BENNETT, 1958). The ChE activity of the tissue sample is reported in terms of moles ACh x 10^{10} hydrolyzed per minute per milligram of tissue. While this analysis does not distinguish between acetylcholinesterase and pseudocholinesterase, it appears unlikely that variations in the pseudocholinesterase activity contribute significantly to the measures we have obtained. To measure the pseudocholinesterase activity toward acetylcholine of homogenates of cortical and subcortical brain, we have used a selective inhibitor of acetylcholinesterase 1:5-bis-(4-trimethylammoniumphenyl)pentan-3-one diiodide⁴ (BAYLISS and TODRICK, 1956; ELKES and TODRICK, 1955). The results indicate that less than 5 per cent of the activity of the tissue homogenate used can be attributed to pseudocholinesterase.

The primary data thus consist of total brain weight and of three measures of ChE activity: (1) ChE in the visual area of the cortex; (2) ChE in the somesthetic area of the cortex; and (3) ChE in the total brain minus the dorsal surface of the cortex--referred to hereafter as the "subcortical ChE activity." Since high correlations have been consistently found between ChE activity in various areas of the cerebral cortex (ROSENZWEIG, KRECH and BENNETT, 1958), the ChE activities of the visual and somesthetic areas have been averaged to obtain a single index of "cortical ChE activity." The three measures of ChE activity are then reduced to two: cortical, and subcortical ChE activity.

RESULTS

Cortical cholinesterase activity. Table 1 presents the data for cortical ChE activity for rats of the S₁ and S₃ strains from 28 to 500 days

Table 1. -- MEAN, STANDARD DEVIATION, AND RANGE OF CORTICAL CHOLINESTERASE ACTIVITY, AS A FUNCTION OF AGE, FOR S₁ AND S₃ STRAINS OF RATS.

| S ₁ | | | | | S ₃ | | | | |
|----------------|-------------|-----------------------|------|-----------|----------------|-------------|-----------------------|------|-----------|
| N | Age in Days | Mean ChE V+S* 2 | S.D. | Range | N | Age in Days | Mean ChE V+S* 2 | S.D. | Range |
| 5 | 28 | 49.6 | 3.49 | 44.9-54.3 | 4 | 29 | 45.8 | 3.63 | 42.2-51.4 |
| 3 | 34 | 55.3 | 1.19 | 53.8-56.7 | 4 | 32 | 50.5 | .83 | 49.4-51.6 |
| 8 | 44 | 58.4 | 5.37 | 48.5-64.8 | 9 | 43 | 57.2 | 3.89 | 54.0-67.6 |
| 9 | 62 | 64.2 | 2.84 | 61.4-69.2 | 8 | 64 | 58.2 | 3.15 | 53.8-63.1 |
| 9 | 82 | 63.0 | 3.06 | 58.0-68.4 | 7 | 81 | 60.6 | 4.60 | 50.6-64.8 |
| 27 | 96 | 63.5 | 4.35 | 55.0-75.0 | 14 | 96 | 57.7 | 3.28 | 53.6-65.0 |
| 50 | 108 | 65.3 | 3.95 | 58.6-72.4 | 54 | 110 | 59.6 | 3.47 | 52.7-69.0 |
| 56 | 118 | 62.8 | 3.16 | 56.2-69.7 | | | | | |
| 50 | 128 | 64.4 | 3.83 | 52.0-72.8 | 41 | 126 | 57.2 | 3.45 | 51.2-63.6 |
| | | | | | 17 | 131 | 60.0 | 2.92 | 55.3-64.6 |
| 13 | 147 | 61.8 | 3.95 | 55.0-73.0 | 6 | 147 | 56.5 | 2.14 | 52.4-58.4 |
| | | | | | 3 | 171 | 56.7 | 5.73 | 50.0-64.0 |
| 5 | 245 | 63.9 | 3.06 | 59.2-68.4 | 6 | 244 | 55.3 | 2.14 | 51.3-58.0 |
| | | | | | 11 | 260 | 58.2 | 5.49 | 51.0-67.0 |
| 8 | 370 | 60.4 | 4.32 | 55.7-68.0 | 3 | 376 | 58.1 | 2.73 | 54.2-60.1 |
| 2 | 500 | 58.5 | 2.50 | 56.0-61.0 | 6 | 527 | 55.0 | 1.29 | 53.0-57.0 |

* Cholinesterase activity was averaged for samples of visual and somesthetic regions of cerebral cortex and is expressed in moles ACh x 10¹⁰ hydrolyzed per minute per mg. of tissue.

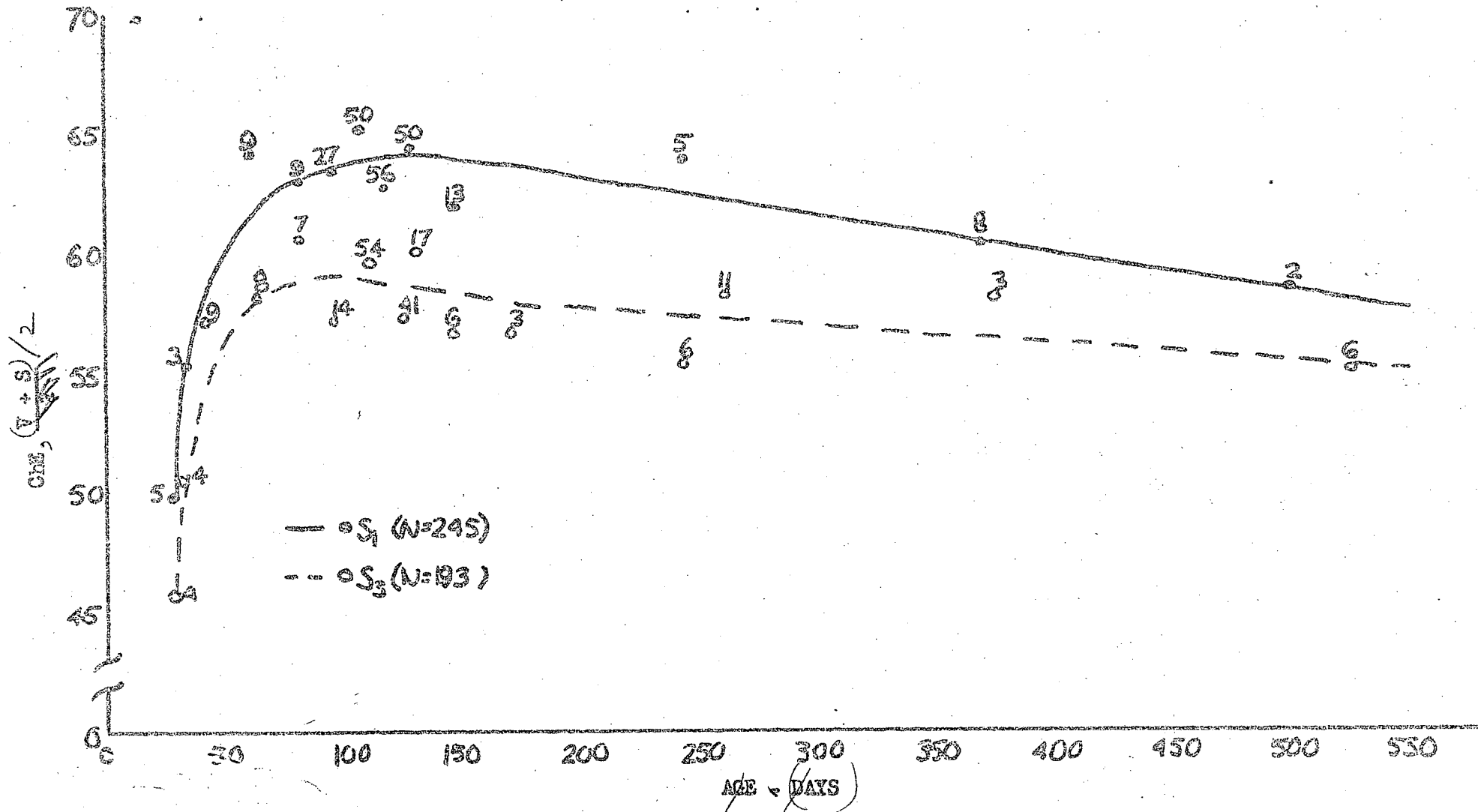


Fig. 1. Cortical cholinesterase (ChE) activity of the S1 and S3 strains of rats as a function of age.
 The ChE values are based on an average of samples obtained from the visual and somesthetic areas of the cortex. They are expressed in moles ACh x 10¹⁰ hydrolyzed per minute per mg. of tissue. The number at each point shows the number of animals upon which the value is based.

of age. During three years of analytical work, data have been obtained for 245 S₁ rats and 193 S₃ rats. In preparing the table, data have been grouped together from all animals sacrificed within a 10-day interval of age, each interval starting with an integral multiple of 10; i.e. 20-29 days, 30-39, 40-49, and so on. For each age group the mean age and the mean, standard deviation and range of the ChE values were computed. Certain groups were very close to each other in age and ChE values, and therefore the number of groups was reduced by combining adjacent ones when their mean ages did not differ by more than 10 per cent and when their mean ChE values did not differ significantly at the 5 per cent level of confidence. When groups were combined, the tabulated statistics represent the whole group.

The changes in cortical ChE activity with age are shown graphically in Fig. 1. The ChE values for both strains rise sharply until about 60 days, and then they continue upward more gently, reaching a broad peak in the region of 100 days. COHN and RICHTER (1956) reported that the peak is reached at 60 days, but a personal communication from Dr. COHN makes it clear that they used few animals older than 60 days. After 100 days our values decline slowly and continuously, subsiding at 500 days to about the level they had at 40 days.

At every age the mean ChE values for the S₁ strain are higher than those for the S₃ strain, the mean difference being about 10 per cent. An analysis of variance was done in order to determine whether the difference in ChE between strains is statistically significant. Since the numbers of cases in the various subgroups differ widely, the technique of analysis of variance for disproportionate subclasses was used (SNEDECOR, 1956). The difference between the two strains was found to be highly significant (1 and 393 df; F=181.5; P < .001). The variance due to differences in age was also highly significant (15 and 393 df; F=12.3; P < .001). The interaction was not

significant, indicating that the shapes of the growth curves cannot be distinguished for the two strains.

The statistics on variability--the standard deviation and range--make it possible to characterize the variability among individual rats in cortical ChE activity. The relative variability can be expressed by the coefficient of variation [$V=(100 \text{ S.D.})/\text{mean}$]. When V is computed for each of the subgroups, the average value for the S₁ rats is 5.70 and for the S₃ rats, 5.73. These coefficients indicate that the individual values tend to cluster rather closely around the means of their groups, two-thirds of the animals having values that differ by less than 5.7 per cent from the mean of their group.

Subcortical cholinesterase activity. Table 2 presents the data for subcortical ChE activity for the two strains. Subcortical ChE activity has been analyzed only during the past year and a half, and values are given for 140 S₁ rats and 135 S₃ rats, distributed over the age range of 28 to 131 days. The data were grouped according to age and combined as in the case of the cortical ChE data.

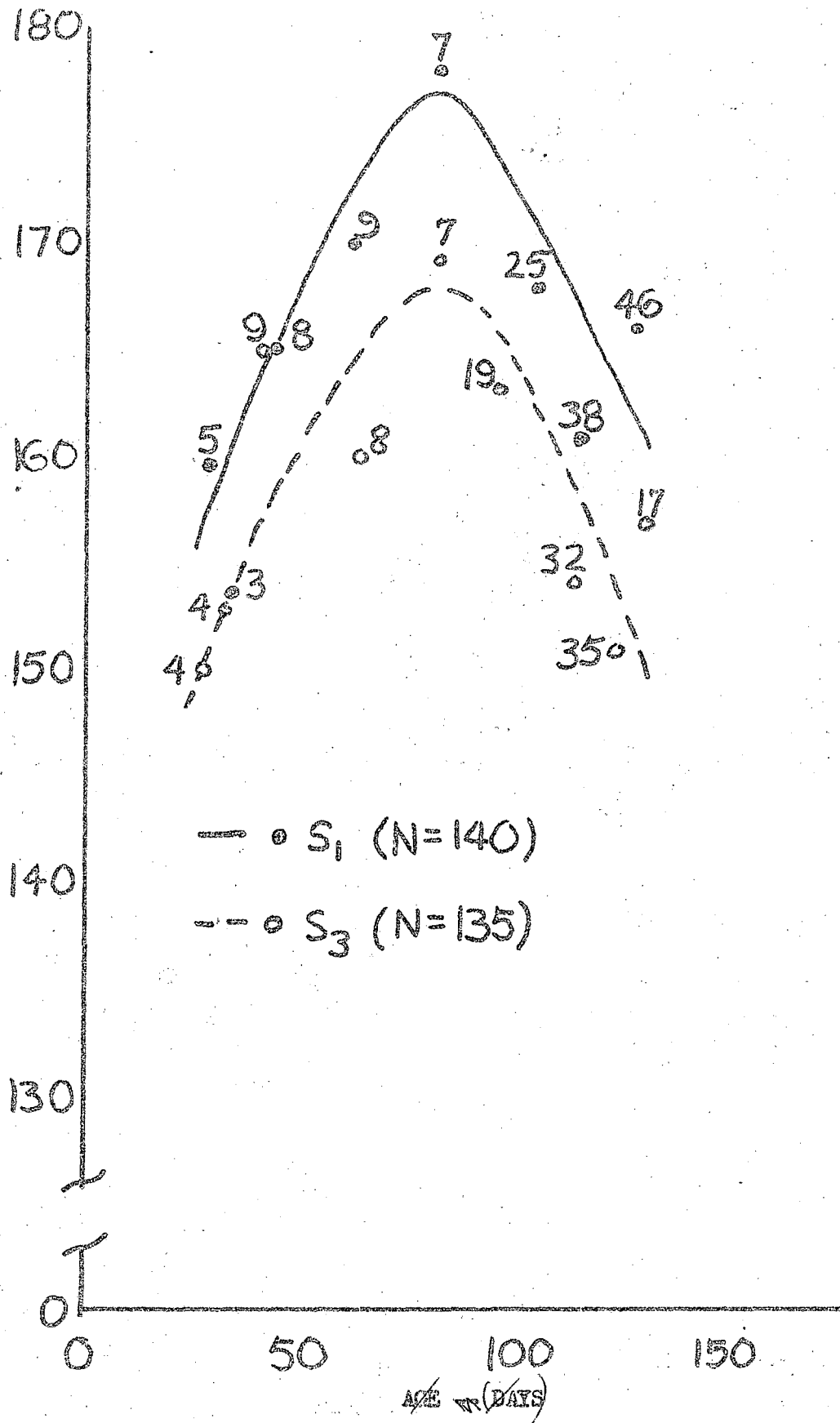
The age functions of subcortical ChE activity are shown in Fig. 2. The curves for both strains show rather sharp peaks at about 80 days. This is in keeping with the finding reported by several workers (ELKES and TODRICK, 1955; COHN and RICHTER, 1956) that subcortical centers reach their maximum ChE activity at an earlier age than does the cortex. Overall, the S₁ values exceed the S₃ values by about 6 per cent. An analysis of variance for disproportionate subclasses was performed to test the significance of the observed differences. The difference in favor of the S₁ strain was found to be highly significant (1 and 258 df; $F=66.7$; $P<.001$). The variance due to differences in age was also significant (8 and 258 df; $F=8.0$; $P<.001$). The interaction was also significant, the two strains differing more at the

Table 2. -- MEAN, STANDARD DEVIATION, AND RANGE OF SUBCORTICAL CHOLINESTERASE ACTIVITY, AS A FUNCTION OF AGE, FOR S₁ AND S₃ STRAINS OF RATS.

| S ₁ | | | | | S ₃ | | | | |
|----------------|-------------|-----------|------|---------|----------------|-------------|-----------|-------|---------|
| N | Age in Days | Mean ChE* | S.D. | Range | N | Age in Days | Mean ChE* | S.D. | Range |
| 5 | 28 | 158.2 | 8.33 | 144-169 | 4 | 29 | 150.0 | 7.58 | 142-159 |
| 3 | 34 | 153.7 | 3.09 | 151-158 | 4 | 32 | 153.0 | 2.12 | 150-155 |
| 8 | 44 | 165.4 | 3.50 | 161-173 | 9 | 43 | 165.2 | 8.51 | 153-184 |
| 9 | 62 | 170.3 | 8.41 | 154-186 | 8 | 64 | 160.1 | 5.88 | 150-167 |
| 7 | 80 | 178.4 | 8.91 | 163-199 | 7 | 81 | 169.3 | 9.05 | 159-185 |
| 25 | 104 | 167.9 | 9.72 | 154-192 | 19 | 98 | 163.1 | 9.28 | 148-182 |
| 38 | 114 | 161.8 | 9.16 | 137-180 | 32 | 114 | 154.8 | 11.91 | 134-180 |
| 45 | 128 | 167.3 | 7.65 | 149-182 | 35 | 127 | 151.2 | 6.76 | 140-164 |
| | | | | | 17 | 131 | 157.4 | 8.59 | 142-171 |

* Cholinesterase activity is expressed in moles ACh x 10¹⁰ hydrolyzed per minute per mg. of tissue.

SUBCORTICAL ChE



— • S₁ (N=140)
 - - • S₃ (N=135)

Fig. 2. Cholinesterase (ChE) activity in the subcortical brain of the S₁ and S₃ strains of rats as a function of age. The ChE values are for brains from which dorsal cortex had been removed. They are expressed in moles ACh x 10¹⁰ hydrolyzed per minute per mg. of tissue. The number at each point shows the number of animals upon which the value is based.

later than at the earlier ages (7 and 258 df; $F=3.0$; $P<.01$).

The coefficients of variation tend to be somewhat smaller for subcortical than for cortical ChE activity. They average 4.42 for the S_1 groups and 4.88 for the S_3 groups. Thus the rats show even less relative variability in subcortical than in cortical ChE activity.

Brain weight. Table 3 presents weights of the total brain for rats of the two strains. Brain weights are available for 142 S_1 rats and 147 S_3 rats, distributed through the age range of 28 to 148 days. Data were grouped and combined as in the case of the ChE data.

The age functions in Fig. 3 rise monotonically. Contrary to the case of the ChE activity data, here it is the S_3 strain whose values are the greater at every age tested. An analysis of variance, done for disproportionate subclasses, showed that the strain difference was again highly significant (1 and 260 df; $F=224.4$; $P<.001$). The age differences were also highly significant, as would be expected for sharply rising functions (9 and 260 df; $F=40.8$; $P<.001$). The interaction is not significant, the vertical distance between the curves for the two strains increasing only slightly with age.

The coefficients of variation are somewhat smaller for brain weight than for the ChE activity measures. They average 3.80 for brain weights of the S_1 strain and 4.45 for the S_3 strain.

DISCUSSION

It is clear from the results that differences in brain chemistry and brain weight are associated with differences in the age and strain of rats. The analyses of variance demonstrated the statistical significance of these sources of variance. It is perhaps worth considering whether the differences among animals of a given age and strain are also significant or whether they

Table 3. -- MEAN, STANDARD DEVIATION, AND RANGE OF BRAIN WEIGHT,
AS A FUNCTION OF AGE, FOR S₁ AND S₃ STRAINS OF RATS.

| S ₁ | | | | | S ₃ | | | | |
|----------------|-------------------|-------------------------|------|-----------|----------------|-------------------|-------------------------|-------|-----------|
| N | Age in Days | Mean Weight (mg.) | S.D. | Range | N | Age in Days | Mean Weight (mg.) | S.D. | Range |
| 5 | 28 | 1255.0 | 44.4 | 1197-1310 | 4 | 29 | 1422.2 | 49.2 | 1380-1502 |
| 3 | 34 | 1386.0 | 36.1 | 1347-1434 | 4 | 32 | 1398.8 | 46.4 | 1325-1453 |
| 8 | 44 | 1506.8 | 60.0 | 1403-1563 | 9 | 43 | 1542.7 | 55.3 | 1453-1620 |
| 9 | 62 | 1464.8 | 58.8 | 1357-1582 | 8 | 64 | 1642.4 | 93.8 | 1516-1746 |
| 7 | 80 | 1557.9 | 65.0 | 1480-1644 | 7 | 81 | 1635.8 | 116.2 | 1439-1803 |
| 11 | 94 | 1630.9 | 73.3 | 1510-1770 | 12 | 96 | 1704.5 | 70.6 | 1601-1840 |
| 15 | 105 | 1575.6 | 55.6 | 1436-1644 | 42 | 112 | 1710.6 | 72.0 | 1562-1842 |
| 71 | 120 | 1600.1 | 65.4 | 1439-1736 | 55 | 128 | 1766.8 | 82.5 | 1600-1940 |
| 8 | 133 | 1657.1 | 63.7 | 1570-1770 | | | | | |
| 5 | 148 | 1638.2 | 61.6 | 1543-1721 | 6 | 147 | 1796.5 | 69.0 | 1657-1859 |

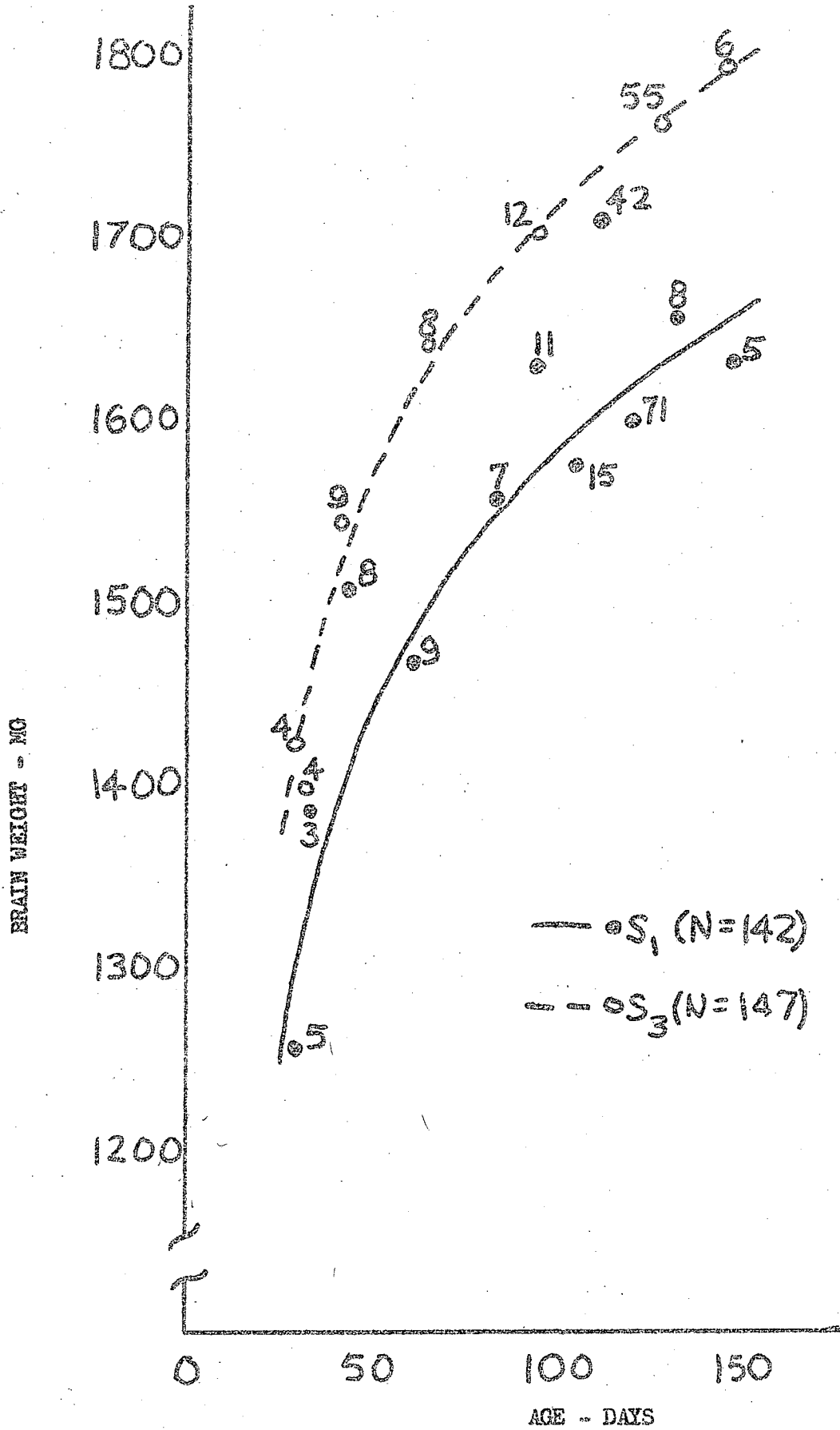


Fig. 3. Brain weight of the S₁ and S₃ strains of rats as a function of age.
 The entire brain was used, including the cerebellum and the olfactory bulbs.

may be attributed to errors of measuring brain weight and ChE activity.

The brains are weighed with less than 10 mg. of error, the error being due primarily to differential loss of water. Tests have shown that variations in techniques of removal of the brain could account at the most for 20 mg. of error. Such errors could certainly not account for the differences of 300 mg. or more found between brains of rats of the same strain and age; therefore it is clear that the observed within-group differences are primarily due to true individual differences.

Two ChE determinations are usually made on each tissue sample, and the mean is taken. Differences between the two analyses of a given tissue sample average about 2 units of ChE activity for the cortex and 4 units for the subcortex; taking the average of the two determinations for each of these parts of the brain further reduces error of measurement. Such errors could not account for differences among rats of the same age and strain of 20 units at the cortex or of 40 units in the subcortical brain. The conclusion that there are real individual differences in brain ChE activity is given further support from a genetic experiment in progress in which it has been possible to breed new strains of rats for high and low cortical ChE activity by selecting their sires according to our standard ChE determination techniques (RODERICK, BENNETT and KARLSSON, 1957). Finally, there is the fact that statistically significant correlations have been obtained between our chemical measures and behavioural measures within strains. This is further evidence that the individual differences in brain chemistry (and in behaviour) are reliable; if they were not, the correlations would reflect only random variation, and would not differ significantly from zero.

The variability of the brain is small compared to the variability of other organs. JACKSON (1913) found that the coefficient of variation for brain weight of the rat was smaller than that for any of the 13 other organ

or system weights he measured. Our data indicate that the enzymic variability of the brain is also held under tight control, for the coefficients of variation for ChE activity are only slightly larger than those for brain weight. We have also found comparable coefficients of variation for lactic dehydrogenase activity in the rat brain. Since individual variation in brain chemistry is small in magnitude, though significant, great care is necessary in making determinations that are to measure values for individual subjects.

The shape of the curve for ChE activity of the subcortical brain should approximate that for whole brain. These values, it will be recalled, were determined after the dorsal cortex--about 20 per cent of the weight of the entire brain--was removed. Since the ChE activity of dorsal cortex is about 40 per cent of that of the subcortical brain, the level of the curve for subcortical brain should be reduced by about 12 per cent to give approximate values for whole brain. The most extensive curve in the literature on ChE development in the rat brain is that of METZLER and HUMM (1951) for the "white rat." It extends from the 14-day fetus to the "adult"; there are 51 points (presumably individual rats) over the whole age range. Only 13 points are for ages greater than 28 days, the age at which our curve begins. The curve of METZLER and HUMM shows an abrupt peak at 22 days, then a decline to a plateau which extends from 32 to 75 days and another decline to the 120-day and "adult" level. ELKES and TODRICK (1955) studied development of ChE activity in the whole brain of the "albino rat." They used 50 rats distributed at 3- or 4-day intervals between the third and thirtieth day of life. Their curve is monotonic and shows no sign of the 22-day peak of METZLER and HUMM. They state that the level at 22 days "is approximately 75% of the fully grown level." NACHMANSOHN (1939), using single rats at each point, found a rise in ChE activity between 21 and 35

days, a smaller rise from 35 to 110 days, and then a decline from 110 to two "adult" animals. BAYLISS and TODRICK report in an abstract (1956) that ChE activity increases linearly from the third to the twentieth day and more slowly thereafter. It may be that different strains of rats differ considerably in growth curves of brain ChE activity. However, in the light of all the evidence, we are inclined to believe that ChE activity for whole brain of the rat reaches its peak around the eightieth day of life.

The change of ACh content with age in whole brain is asserted by WELSH and HYDE (1944) to be closely correlated with that of ChE activity. They, and also CROSSLAND (1951), found that ACh doubles from 24 hrs. to 21 days and doubles again by "100+" days. While the direction of change is the same for ACh and ChE, it appears that ChE activity increases more rapidly than ACh up to 21 days and then more slowly than ACh.

Our ChE activity determinations, like those of others, give activity per unit of weight. It is interesting to note that the S₃ strain has equal or slightly larger total ChE activity than the S₁ strain (ChE activity x brain weight). Since the S₃ brains are heavier, this activity is less per unit of weight in the S₃ brain. It is possible that the ChE difference reflects a different ratio of neural to glial structures in the two strains. Such an anatomical difference between strains could have important functional significance. If such an anatomical difference exists, it cannot be uniform throughout the brain, since the ChE difference is relatively larger at the cortex than subcortically. An histological investigation to attempt to determine the source of the strain differences in ChE activity is in progress.

SUMMARY

Cholinesterase (ChE) activity in the cortical and subcortical brain and brain weight were determined for male rats of two strains as a function of age. The S₁ strain was found to have significantly greater cortical and subcortical ChE activity and significantly lower brain weight than the S₃ strain. Significant effects of age were also found for each variable. It was shown that for animals of a given strain and age, there are true individual differences that cannot be attributed to errors of measurement. Thus the rat's brain ChE activity and brain weight differ according to genetic background, age, and individual factors.

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