# Serum Arylesterase Levels of Activity in Twins and Their Parents

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Little is known about the factors controlling the activity of the enzyme arylesterase (E.C. 3.1.1.2) in human serum. There is no known natural substrate. The enzyme optimally hydrolyzes phenylacetate; its activity is enhanced in the presence of calcium ions, is inhibited by various chelating agents, metal ions, and sulfhydryl reagents [1], and it resists inhibition by alkyl phosphates and eserine [2]. There appear to be at least two types of arylesterase in human serum.

Björklund et al. [3] have separated the esterases by curtain electrophoresis into an EDTA-sensitive, heat-labile type and an EDTA-dependent, heat-stable type. The EDTA inhibition could be prevented by addition of manganese ions. Wilde and Kekwick [4] found three arylesterases after preparative electrophoresis on cellulose acetate; one migrated to the prealbumin region, one was associated with the albumin, and the third was associated with the cholinesterase. The first two had *p*H optima of 7.9, and the cholinesterase fraction had a *p*H optimum of 8.5. The enzyme in the prealbumin fraction had a preference for the acetyl group, the one in the albumin fraction showed no specificity for an acyl group, and the arylesterase of the cholinesterase fraction had a preference for the butyryl group. The enzyme in the prealbumin fraction was the only one which was sensitive to reagents for thiol groups and required $Ca^{2}+$ ions for activation.

Arylesterases have been reported in other human tissues. There are probably three forms in red blood cells [5] and at least three in cells grown from cervical carcinoma, amnion, and normal cervix [6].

Marton and Kalow [7] reported a considerable variation in the activity of arylesterase in human sera as measured by phenylacetate hydrolysis among 74 individuals who were mentally ill. The activity of the enzyme was not correlated with that of serum cholinesterase except in an additional 25 patients with cancer [7]. The present study has been concerned with an attempt to identify the factors which control arylesterase activity in human sera, with particular emphasis on the recognition of genetic control.

## SUBJECTS AND METHODS

### *Population*

The population consisted of 53 pairs of twins who had volunteered for a psychological study and 35 pairs of their parents. There were 81 males and 95 females, all considered

Received October 29, 1970; revised December 29, 1970.

This work was supported by MRC grant MT 1876.

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to be healthy. Most of the twins were asked to join the study through their schools and were therefore between the ages of six and 18 years, but some were adult twins who responded to a request in the local press. One-half of the twins were selected because they were like-sexed, and 47 pairs were actually like-sexed.

*Zygosity of the Twins*

The zygosity of the twins was based on eight blood groups (typed by B. P. L. Moore of the Canadian Blood Transfusion Service, Toronto), atd angles, and total ridge counts. In cases where the probability of being monozygotic (MZ) was less than .98 and there was one difference in the blood groups for dizygotic (DZ) twins, Hp, Gc, and two ChE types were tested. The probability of zygosity was calculated using the method of Smith and Penrose [8].

*Measurement of Activity of Arylesterase*

Serum from clotted blood was used for all assays. Each sample had been frozen and thawed at least twice and stored at −20° C. The activity was lowered by the first two freezings and thawings and then remained rather stable up to the fifth or sixth freezing and thawing and after several months of storage. The arylesterase activity was a measure of the change in absorbance at a wavelength of 269 m$\mu$ which occurred during two minutes at 26° C using a Beckman recording spectrophotometer (model DK2) according to a modified method of Marton and Kalow [7]. The final dilution of the crude serum was 1:400. The units were the recorded change in the optical density (OD) units ($\times$ 10 for convenience) for the two-minute period under the above conditions. Each assay was repeated in duplicate on two different days, making a total of four repetitions. Since no EDTA was added, the EDTA-sensitive, heat-labile (activity disappeared at 56° C) enzyme as Björklund et al. [3] described was presumably being measured.

Since cholinesterase (E.C. 3.1.1.8.) is known to hydrolyze phenylacetate slightly [2], an experiment was done to ensure that hydrolysis of the substrate by cholinesterase was not being measured by the assay for arylesterase. The addition of $1.25 \times 10^{-5}$M of eserine to six sera in a 1:400 dilution completely inhibited the activities of cholinesterase using the method described by Kalow and Lindsay [9] with benzoylcholine as substrate. The activity for arylesterase from the same sera was then measured in a 1:400 dilution using phenylacetate as substrate with and without the addition of $1.25 \times 10^{-5}$M of eserine. The activity of the enzyme was measured in sera from one person taken on three different occasions over a period of four months.

*Inhibition Studies*

The activity with and without $2 \times 10^{-3}$M $MgCl_2 \cdot 6\ H_2O$ was measured for sera from 15 individuals randomly chosen from members of the twin families. The activity was also measured with and without $9 \times 10^{-2}$M $MgCl_2 \cdot 6\ H_2O$ for sera from the same 15 individuals plus eight additional members of the families.

*Statistical Analysis*

The mean enzyme activity and age were calculated for both sexes, the regression coefficient for the activity versus age, and analysis of variance for the enzyme activity between and within families. For the correlation coefficients of arylesterase activity between pairs of relatives, if one of the parameters were children, the MZ twins were treated as one individual and their mean activity was used. The confidence limits of the correlation coefficients were calculated using a $z$ transformation or, when $N$ was less than 50, a $z^*$ transformation, as described by Sokal and Rohlf [10].

## RESULTS

### *Zygosity of Twins*

There were 25 pairs of MZ and 28 pairs of DZ twins. The probability for twins being MZ was .98 or greater and in most cases was .99 or greater. For each pair of DZ twins, there were differences in at least two of the blood groups or serum markers.

### *Reliability of the Enzyme Assay*

There was no change in the rate of hydrolysis with or without eserine when phenylacetate was used as substrate, indicating that no cholinesterase was hydrolyzing the phenylacetate at the serum dilution of 1:400.

### *Range and Means of Arylesterase Activity*

The activity measured from sera taken from one individual on three different occasions was reasonably constant. The standard deviation was $\pm$ 30 units for the means of the determinations of each of the three samples. The units of activity varied from 205 to 1,162 units in the sera from the 176 individuals and followed a unimodal distribution somewhat skewed to the right (fig. 1). The mean levels of activity by age and sex are summarized in table 1. The mean ages for males and females were 30 years $\pm$ a SE of 2 and 28 years $\pm$ 2, respectively. The mean activity of the enzyme was 534 units $\pm$ a SE of 20 for males and 533 units $\pm$ 19 for females. The activity was not apparently affected by the sex of the individual.

### *Inhibition Studies*

The inhibition of arylesterase activity by $2 \times 10^{-3}$M $Mg^{++}$ ranged from 17%–32% with no correlation of percentage inhibition and activity. The inhibition for $9 \times 10^{-2}$M $Mg^{++}$ ranged from 93%–97% and, again, was not related to activity. Some sera whose activity was near the limits of the high and low ranges were included in the inhibition studies.

### *Variation of Arylesterase Activity with Age*

Since there was no sex difference, the data for sex were pooled when testing for a relationship between enzyme activity and age. Arylesterase activity rose gradually with age (fig. 2). Although there was a wide range of enzyme activity for various ages, the correlation coefficient of 0.3 was significantly greater than zero ($t = 4.17$ for 174 df, $P < .01$). Therefore, a correction was applied to each measure of arylesterase for all subsequent analyses. The correction was as follows: units $+ b \times$ (mean age — actual age) where $b$ was 3.18, the regression coefficient.

### *Family Data*

The variance for arylesterase activity was greater between families than within families (table 2). This result suggests that there are factors common to families

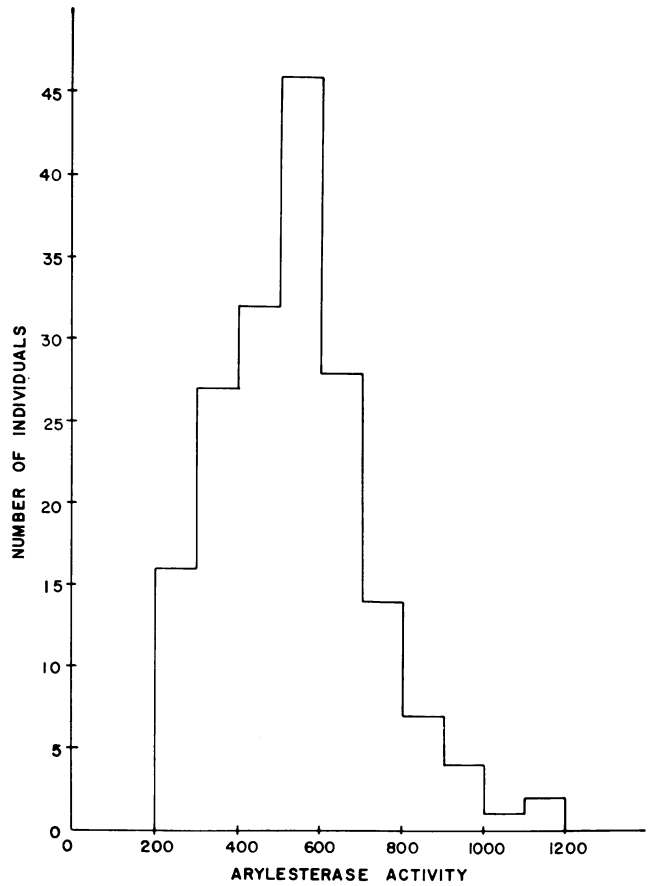


FIG. 1.—Distribution of arylesterase activity in arbitrary units (see text) for 176 individuals

TABLE 1

ARYLESTERASE ACTIVITY IN SERA FROM MALES AND FEMALES

| SEX | No. PERSONS | ARYLESTERASE ACTIVITY Mean s.e. | AGE Mean s.e. |
|---|---|---|---|
| Male ..... | 81 | 534 ± 20 | 30 ± 2 |
| Female ..... | 96 | 533 ± 19 | 28 ± 2 |

which cause the enzyme activities to be similar within them; however, the factors are not necessarily hereditary.

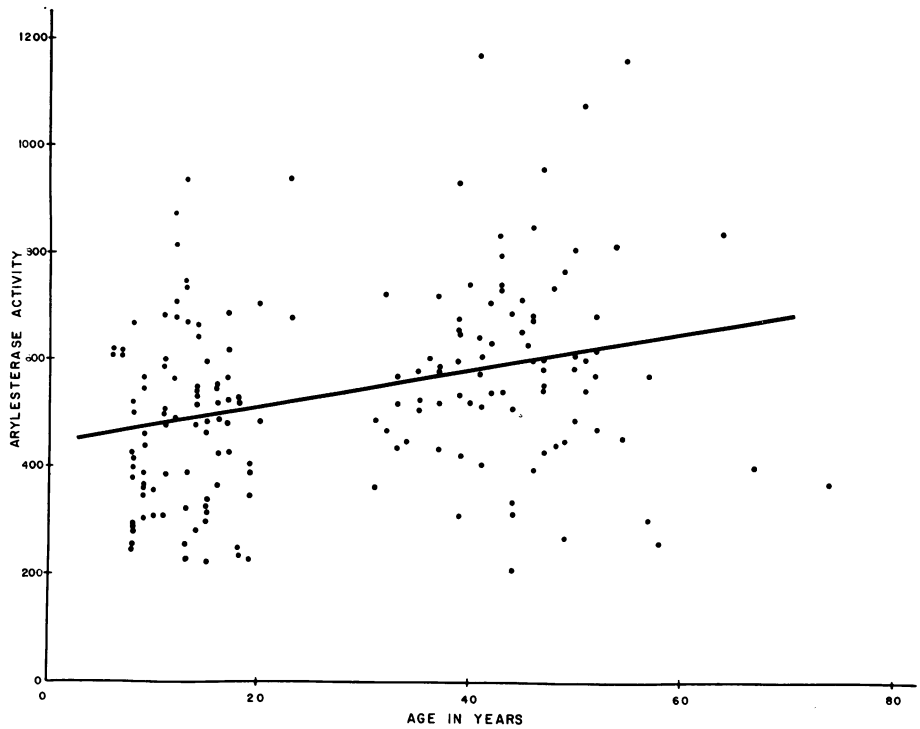


FIG. 2.—The gradual rise of arylesterase activity with age; regression coefficient, 3.2

TABLE 2

ANALYSIS OF VARIANCE FOR FAMILY DATA ON ARYLESTERASE ACTIVITY

| Source of Variation | df | Sum of Squares | Mean Squares | *F* |
|---|---|---|---|---|
| Between families ..... | 34 | 2,244,624 | 66,018 | 3.41** |
| Within families ..... | 105 | 2,030,832 | 19,341 | ... |
| Total ..... | 139 | 4,275,456 | ... | ... |

** $P < .01$.

If a characteristic were entirely under genetic control, the correlation coefficients theoretically would be 1 for MZ twins, .5 for DZ twins, and 0 for parent pairs. If the genetic control were not 100%, and if the assumption is made that environmental components are the same for both types of twins, the difference between the correlation coefficients for MZ and DZ twins is an estimate of half of the heritability if the genetic control is due to additive genes [11]. If some genetic

control were due to dominance, the difference between the correlations would be reduced; hence, the difference can be considered as an estimate of the upper limit of half the heritability.

The correlation coefficients for MZ twins, DZ twins, and parent pairs are given in table 3. The difference between the correlations for arylesterase activity in MZ and DZ twins is .37; hence, the estimate of the upper limit of heritability is 74%. If all the resemblance between parent pairs were due to common environment, the

TABLE 3

CORRELATIONS BETWEEN RELATIVES FOR ARYLESTERASE ACTIVITY

| Relationship | No. Pairs | Correlation Coefficient | 95% Confidence Limits† |
|---|---|---|---|
| Parent-child | 108 | .32 | .15–.48 |
| Mother-child | 55 | .44 | .20–.63 |
| Father-child | 53 | .19 | —.08–.44 |
| Mother-daughter | 29 | .45 | .27–.57 |
| Mother-son | 26 | .47 | .28–.60 |
| Father-daughter | 29 | .24 | .05–.40 |
| Father-son | 24 | .20 | —.02–.38 |
| Mid-parent–child | 53 | .41 | .16–.62 |
| MZ twin-twin | 25 | .85 | .76–.89 |
| DZ twin-twin | 28 | .48 | .30–.60 |
| Parent-parent | 35 | .27 | .10–.42 |

† Calculated using $z$ or $z^*$ (for $N \leq 50$) transformation [10, p. 518].

correlation of .28 for arylesterase in parent pairs agrees reasonably well with the twin data which suggested that about 26% of the resemblance between twins was due to nongenetic factors. However, when the confidence limits of the correlations for the parent and DZ twin pairs are considered, they overlap.

The correlation coefficients for other pairs of relatives are given in table 3. Theoretically, parent-child, mother-child, and father-child correlations should be the same as that for DZ twins, and mid-parent–child would be expected to be greater [12]. As can be seen from table 3, the above correlations are similar to each other since the confidence limits cover wide ranges but, again, they are not different from the parent-parent correlation. The father-child correlation may be lower than the others, although not significantly different for this small sample. The father-child correlations appear to be lower for both father-daughter and father-son pairs as compared with those for mother-daughter and mother-son pairs (table 3). However, the confidence limits are so large that the differences could have occurred by chance.

## DISCUSSION

Augustinsson and Brody [13] have shown that the activity of arylesterase was much lower in newborn infants than in normal adults and that the activity did not

increase after two years of age [14]. In the present study, all of the individuals were over six years of age, and the level of arylesterase activity gradually increased with age.

The mean levels of enzyme activity in men and women for the present study were the same. Augustinsson and Barr [14] also did not find any sex differences in the rate of increase of the levels of the enzyme activity in human sera. For other animals, however, the levels of enzyme activity have been altered by sex hormones. Arylesterase activity was lowered by a male sex hormone in pigs [15] and in dogs [16], and by estradiol in rats [17]. Augustinsson and Henricson [18] reported a sex difference in rats; enzyme activity levels were higher in females.

Arylesterase activity has been shown to be genetically determined in two species. The enzyme in pig serum has similar properties to arylesterase in human serum [1]. Its activity in pig serum has been shown to be controlled by a series of five alleles, each determining zero, 25, 50, 75, and 100 units of activity with additive effects [19]. The products of two alleles recognized by differences in their migration after electrophoresis in rabbits showed differences in the activity of the enzyme [20]. In rats, there is a genetically controlled variant recognized by electrophoresis which is not related to the activity of the enzyme [21].

In the present study, there was evidence from the twin data that arylesterase activity in man is under genetic control, although there were also unknown nongenetic factors related to the enzyme activity. The influence of age had already been removed (to some extent, at least) by the correction. Because the unknown nongenetic factors contributed substantially to the level of enzyme activity, correlations for DZ twins and parent-child combinations for the limited sample were not significantly different from the correlation for parent pairs and could not be interpreted.

The twin data strongly suggest that there is some type of genetic determination of the enzyme activity, but so far, attempts to find a discrete variant by inhibition studies and by starch and acrylamide gel electrophoretic studies have failed. During electrophoresis of crude serum at *p*H 8.6, 7.4, and 5.3, the enzyme migrates with the albumin, and most coupling dyes stain the albumin when no substrate is present [22]. Hence, unless a variant isozyme migrated independently from the albumin as the faster moving zone in sera from rats [21], it would not be detected.

## SUMMARY

The distribution of arylesterase activity was unimodal and somewhat skewed to the right for 176 individuals. There was no difference in enzyme activity between sexes, but the level of activity gradually rose with age. Measurements of arylesterase in 28 pairs of dizygotic twins and 25 pairs of monozygotic twins indicated that heritability for the enzyme activity was 74%. Data from parent pairs indicated that in addition to genetic and age factors, unknown nongenetic factors substantially affected enzyme activity. Conclusions regarding the extent of genetic control from additive genes and dominance were not possible. No discrete variant related or unrelated to enzyme activity was found.

ACKNOWLEDGMENTS

We are grateful to Dr. G. J. S. Wilde and Mr. G. Kluger of the Psychology Department, Queen's University, for obtaining the cooperation of the families, and to Dr. B. P. L. Moore of the Canadian Red Cross Blood Transfusion Service for blood typing.

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