

Genetics of IgE and allergy: Serum IgE levels in twins

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Serum IgE levels are an important risk factor in allergic disease resulting from immediate-type hypersensitivity. IgE levels of middle-aged male monozygous twins (54 pairs) and dizygous twins (39 pairs) were measured and a significant genetic effect on IgE levels was observed ($H = 0.592$). A significant genetic effect was also noted in twin children ($H = 0.789$). The statistical relationship between IgE levels and atopic disease, as well as aspects of the methods of analysis of twin data, is discussed.

Immunoglobulin E (IgE) of heterogeneous specificity is normally present in human serum, and a portion of the IgE, especially that of allergic individuals, reacts with identifiable environmental antigens.^{1, 2} IgE binds to mast cells in the skin and elsewhere, and to basophilic leukocytes, thereby sensitizing these cells to release pharmacologically active mediators when they are subsequently exposed to antigens to which the IgE is specifically reactive. The occurrence of atopic disease as a consequence of this mechanism is contingent upon several factors, including the quantity of sensitizing IgE,¹ specific characteristics of the target organ, and environmental exposure to allergens.

The immunologic response of individuals exposed to similar environments is not uniform, and a wide range of serum IgE levels has been reported in unselected populations of human adults.³⁻⁸ In the studies in which appropriate data are available, the distribution of values appears to be multimodal.⁵⁻⁹ These observations raise the possibility that IgE levels are determined largely by genetic factors for which the populations studied are polymorphic.⁸⁻¹¹

The familial incidence of atopic disease is high; most family studies report a positive family history in approximately 50 per cent of cases.¹²⁻¹⁴ However, the mode of inheritance is disputed and single dominant gene with partial penetrance,^{12, 13} single recessive gene with partial penetrance,^{15, 16} and multigenic¹⁷ inheritance have been suggested. It has not been possible, with the data available, to distinguish between these alternatives.¹⁴ If the inheritance of atopic disease is multifactorial, it may reasonably be assumed that serum IgE levels are one relevant variable. Consequently, the analysis of the genetics of

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serum levels of IgE may be expected to be less complicated than the analysis of allergic disease in general.

The extent of genetic influence on a continuously varying characteristic, such as serum IgE level, may be estimated by a comparison of the mean intrapair variance of monozygous twin pairs with the mean intrapair variance of dizygous twin pairs, if it is assumed that environmental and other nongenetic influences on the two types of twins are equivalent. The limitations of the twin method have been extensively discussed¹⁸⁻²¹; however, it is a practical approach to establishing the existence of genetic influence, especially in the analysis of characteristics for which multifactorial inheritance has been postulated or when there are thought to be large nongenetic effects. We therefore measured the serum IgE levels of a large number of twin pairs and present the results and detailed analyses.

MATERIALS AND METHODS

IgE assay

IgE levels were measured with a radioimmunosorbent assay previously described in detail.⁸ A single lot of secondary standard consisting of lyophilized aliquots of a high-titer serum initially calibrated against World Health Organization Standard 68-341²² was used for generation of standard curves, and all IgE levels are expressed as units relative to serum 68-341; the IgE unit has been reported to contain approximately 2.4 ng. of IgE.^{8, 23, 24} Duplicate assay tubes were run simultaneously for all samples and standards; assays were repeated on occasions when the ¹²⁵I counts in duplicates differed from the mean value by more than 2 per cent. In cases in which two sera constituted a pair, both assays were done within the same run.

The radioimmunosorbent assay used may overestimate the IgE levels in some, but not all, samples.⁸ The resulting errors in most cases are small, but are occasionally as large as 20 U. per milliliter. This nonspecific blocking phenomenon has subsequently been noted in radioimmunoassays of IgE using other antisera, myeloma proteins, and solid-phase supports^{25, 26} and may be a general phenomenon in these assays. However, the very large systematic errors observed by Polmar, Waldmann, and Terry²⁶ were not encountered in this study or in a previous study⁸ and appear to be unique to their procedures.

Errors resulting from nonspecific blocking were minimized by the following method. In groups of sera, consisting of samples of at least 2.0 ml., the IgE levels of samples having less than 25 U. per milliliter were calculated from the differences between the IgE levels measured before and after absorption of the sample with insolubilized anti-IgE. In the case of paired sera, absorptions were done on both sera when the unabsorbed value of one of the sera was 25 U. per milliliter or less and the unabsorbed value of the other serum of the pair was 45 U. per milliliter or less.

An aliquot (1.0 ml.) of the serum to be absorbed was incubated with 0.1 ml. of a suspension of Sepharose-bound rabbit anti-PS IgE and mixed by continuous rotation at room temperature for 18 hours. Under these conditions, essentially all (130 U.) of a 1/10 dilution of high-titer serum initially containing 1,300 U. per milliliter was absorbed and, thus, any residual inhibition in the assay after absorption of experimental serum by this method was attributable to nonspecific effects. Because of the low IgE levels to be measured, 0.4 ml. of serum, both before and after absorption, was assayed. IgE levels were then computed as:

$$(\text{U./ml. in unabsorbed serum}) - (\text{U./ml. in absorbed serum}) (1.1),$$

to correct for the dilution factor of 1.1 introduced by addition of immunosorbent. For the purposes of calculation, levels less than 10 U. per milliliter measured by this method were taken

to be 10.0 U. per milliliter. In groups of plasma, of which limited volumes were available, no absorptions were done and, to preclude excessive variance due to errors at low levels, concentrations of 15 U. per milliliter or below were taken to be 15.0 U. per milliliter.

Samples obtained for use in this study were stored at -15° and were thawed for removal of aliquots and refrozen on several occasions.

Population sampled

A sample consisting of 93 zygosity-typed pairs of twin sera, as well as 9 additional sera for which a paired serum was not available, were acquired through the courtesy of the National Research Council's twin registry. The population from which the samples were drawn consisted of Caucasian male twins, both of whom were American military veterans between the ages of 45 and 55 and were otherwise unselected. The twins were seen at the Ranchos Los Amigos Hospital, Downey, California, during 1972. The characteristics of this population have been previously described.^{27, 28} Blood was drawn from both twins of a pair usually within two weeks (91 per cent of the pairs) and, in most cases, on the same day (80 per cent), with the greatest interval being $2\frac{1}{2}$ months. The zygosity types were based on serotyping, including the ABH, MN, S, P, Rh, Kell, Lewis, Duffy, Kidd, Diego and Rh- systems, and are assumed to give an average accuracy in excess of 96 per cent in identifying monozygous twins. The sera from one pair of monozygous twins discordant for IgE levels were additionally checked for serum protein polymorphisms in Gm a, x, z, g, f, b⁰, and b¹, Inv a, Gc, Or, Gb, and Hp, and were found to be concordant [Gm (a, z, g, f, b⁰, b¹), Inv (a), Gc (2-1), Or (FS), Gb (SS₁), Hp (2-1)]. The typing for these polymorphisms was done by Dr. C. Alper, Center for Blood Research, Boston.

A sample of 23 twin plasmas obtained at the Crippled Children's Division, University of Oregon Medical School, for a variety of purposes unrelated to allergy or immunology, was also studied. Blood from both twins was drawn on the same day. The children were between $2\frac{1}{12}$ and $9\frac{1}{12}$ years of age (MZ, $\bar{x} = 6.52 \pm 1.66$ S.D., $N = 10$; DZ, $\bar{x} = 7.05 \pm 1.70$ S.D., $N = 13$). Both members of each pair studied were of the same sex; 50 per cent of the monozygous pairs and 46 per cent of the dizygous pairs were males. Serotyping was done as for the veteran pairs, but because of the availability of parental serum, the mean probability of correct zygosity determination in monozygous twins was in excess of 99 per cent.

The geometric mean of serum IgE levels of children has been reported to increase by approximately 30 per cent over the period from 5 years of age to adulthood²⁹ and to decrease gradually in middle age.³ However, the slope of the regression of the $\log_{10}$ of IgE levels on age in the group of twin children studied was not significant ($0.0094 \log_{10}$ IgE units per year, S.E. = 0.072); since the ages of the twin children were distributed near the mean age for the group, the variation of the IgE level with age was considered to be negligible relative to the population variance. There was no significant sex difference in IgE levels among the children.

Individuals studied for the temporal stability of serum IgE levels consisted of 11 men and 12 women, largely laboratory personnel whose sera had been acquired and stored as control sera for other studies. The individuals ranged between 17 and 55 years of age and were unselected with respect to general health and allergic or immunologic disease. Neither the interval between the first and second sample nor the time of year in which the first or second sample was drawn was systematically scheduled and, retrospectively, no clear pattern of times is apparent. The times are assumed to be random, although an arbitrary minimum interval of 6.0 months was set. The population consisted of Caucasians, with the exception of two Negroes, whose initial IgE levels were 80 and 2,380 U. per milliliter.

Statistical methods

The $\log_{10}$ of serum IgE levels was taken to be a normally distributed variable. This approximation is evaluated in the section entitled "Results."

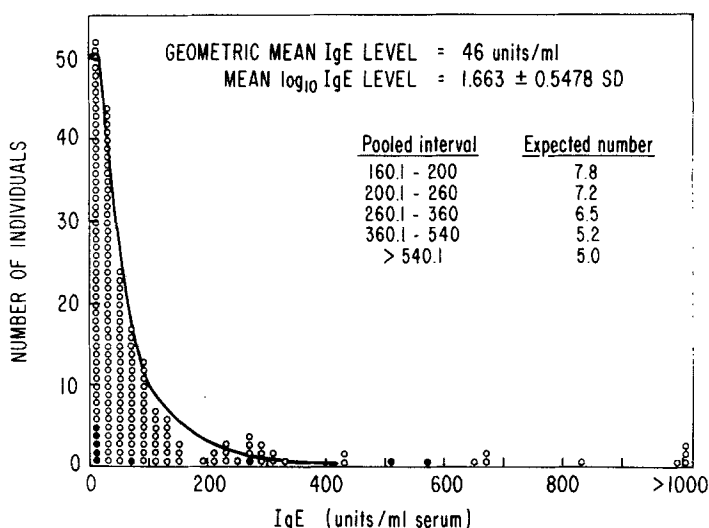


FIG. 1. Distribution of serum IgE levels of 195 veterans. Intervals on the abscissa are of uniform width (20 U. per milliliter); observations and expected numbers falling within the interval are plotted at the midpoint. ○, Veteran twins; ●, veteran twins without paired sera. The curved line shows the expected number computed for a normal distribution with a mean of 1.663 ($\log_{10}$ IgE) and standard deviation 0.5478. The sums of expected numbers of individuals in intervals pooled to contain an expected number of at least 5.0 are given in the chart.

The average degree of dissimilarity between members of a pair in a given category, for example monozygous twins, was computed as the mean intrapair variance¹⁸:

$$V_{MZ} = \frac{\sum (x_i - x_i')^2}{2N},$$

where x_i and x_i' were the $\log_{10}$ of IgE levels of the i th pair of N pairs of monozygous twins, irrespective of which twin of the pair was taken to be x_i or x_i' . Intrapair variances were similarly calculated for dizygous twins (V_{DZ}), measurement error (V_E , where x_i and x_i' were the $\log_{10}$ IgE levels of the same serum in two simultaneous determinations of the IgE level of N randomly chosen serum samples) and for temporal fluctuation (V_F , where x_i and x_i' were $\log_{10}$ IgE levels of sera drawn from a single individual at two different times, there being N individuals in the study population).

The statistical significance of the ratio of two variances was determined from the F table³⁰ or by interpolation in the z table³¹, F calculated as

$$\frac{V_a}{V_b} = F'$$

(where V_a was the larger of the two variances) and having N_a and N_b degrees of freedom. A single-tailed test was used.

Interpair variance is assumed to be the random population variance (V_p)¹⁸ and was calculated, in dizygous twins, as

$$V_{pDZ} = \frac{\sum x_i^2 - (\sum x_i)^2/N}{2N} + \frac{\sum x_i'^2 - (\sum x_i')^2/N}{2N},$$

where x_i and x_i' are $\log_{10}$ IgE levels of the i th pair of dizygous twins and where the members of a twin pair are randomly chosen to be x_i or x_i' . A discussion of this treatment is presented in the *Appendix*. Interpair variance was also calculated by this method for the $\log_{10}$ of IgE levels of sera drawn from a single individual at two different times (V_{pF}). The degrees of freedom associated with V_p were taken to be $N - 1$ for calculations of statistical significance.

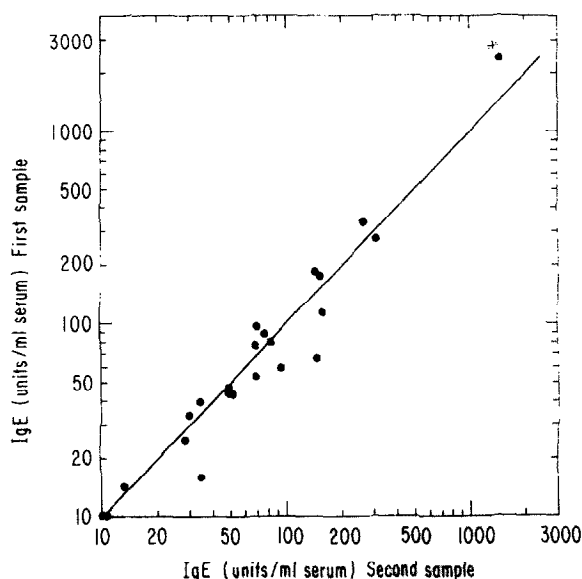


FIG. 2. Serum IgE levels of the same individual at different times. The first of each pair of observations is plotted on the ordinate. The data from an individual with severe allergy are indicated by an asterisk.

To obtain an estimate of the proportion of the variance due to genetic effects, the quantity H was calculated with a small correction for measurement error as

$$H = \frac{V_{DZ} - V_{MZ}}{V_{DZ} - V_E}$$

The 90 per cent confidence interval of H was calculated as

$$P \left\{ 1 - \frac{1 - H}{F_{0.05}} \leq H \leq 1 - \frac{1 - H}{F_{0.95}} \right\} = 0.90$$

where the $F_{0.05}$ and $F_{0.95}$ are respectively the fifth and ninety-fifth percentiles of the F distribution with N_{MZ} and N_{DZ} degrees of freedom.²⁰ Correction of the observed value of H for imperfect zygosity determinations²⁷ was not done.

The regression of $\log_{10}$ IgE levels on age was computed with the mean $\log_{10}$ IgE levels of twin pairs as the dependent variable; the significance of the calculated slope was determined by established methods.³² Other regressions were similarly calculated.

The significance of the differences of means for groups of paired sera was computed by the t test, using the overall average $\log_{10}$ IgE level as the calculated mean. The variance was computed as

$$\frac{1}{2} \left(\frac{\sum x_i^2 - (\sum x_i)^2/N}{N-1} + \frac{\sum x_i'^2 - (\sum x_i')^2/N}{N-1} \right)$$

where x_i and x_i' are the i th of N pairs. For twin sera, x_i and x_i' were assigned randomly to be one or the other serum of the pair, but in the case of a pair of samples from the same individual, x_i was taken to be the (chronologically) first sample. The contribution of the group of N pairs of twins to the total degrees of freedom was taken to be $N-1$, resulting in an underestimate of the significance. The magnitude of the underestimate is dependent upon the correlation between twins of a pair. Confidence limits of Pearson's correlation coefficient (r) and of the intraclass correlation coefficient (r') were computed using the appropriate z transformation.³³

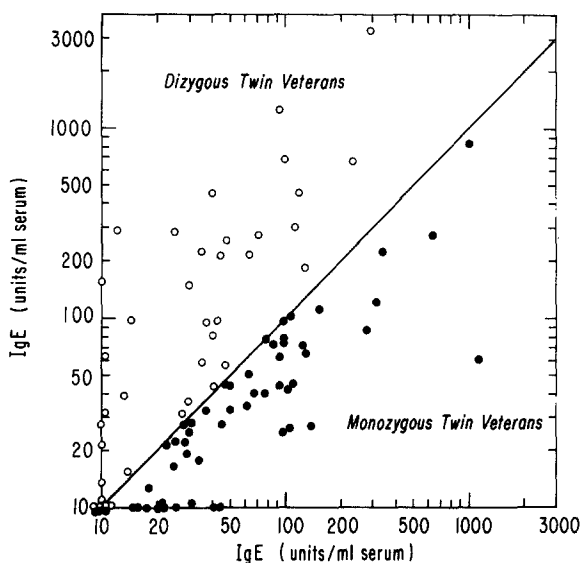


FIG. 3. Serum IgE levels of veteran twins. ●, Monozygotic twins, the higher concentration of each pair plotted on the abscissa; ○, dizygotic twins, the higher concentration of each pair plotted on the ordinate.

RESULTS

The distribution of the IgE levels of the sera from veteran twins, including samples from 9 individuals whose twin was not available, is compared in Fig. 1 to the distribution expected to occur with a normally distributed variable with the same mean $\log_{10}$ IgE level and standard deviation of $\log_{10}$ IgE values. The variance for this calculation was taken to be the weighted average of the variance of all $\log_{10}$ IgE levels of first twins (randomly assigned), of second twins, and of unpaired sera. A χ^2 test of the goodness of fit was done using class intervals 20 U. per milliliter in width, except in cases in which the expected number in a 20 U. interval was less than 5.0. The value of χ^2 was 13.65 with 12 degrees of freedom, and the probability of this value occurring by chance is between 0.5 and 0.25. Thus, analysis of the $\log_{10}$ IgE data by statistical methods for normally distributed variables is not unreasonable. It is apparent, however, that the distribution shows two peaks. Although the peak at approximately 250 U. per milliliter is not sufficiently large to significantly affect the χ^2 value for the distribution as a whole, it suggests a bimodality, despite an overall fit to a log normal distribution.

In order to estimate the variation in IgE levels in single individuals over a period of time, a second serum was obtained from 23 individuals whose serum had previously been stored. The second serum samples from each individual were obtained after an interval of from 6.5 to 63 months ($\Delta \bar{t} = 17.2 \pm 12.0$ S.D.) and were then stored for various intervals until both the first and second sera could be assayed concurrently. The values observed in the first and second

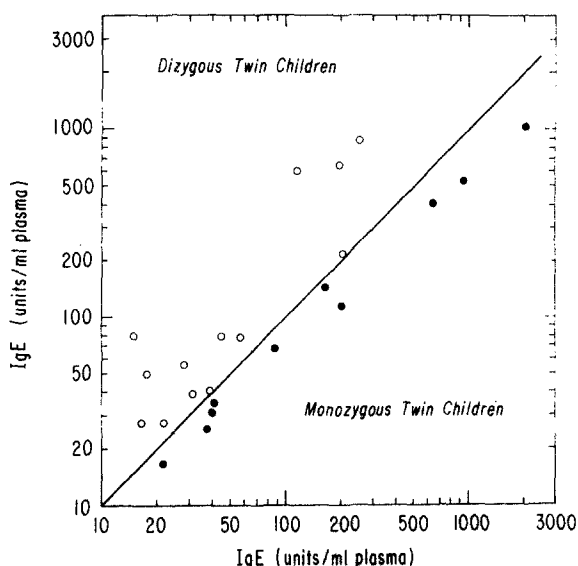


FIG. 4. Serum IgE levels of twin children. •, Monozygotic twins, the higher concentration each pair plotted on the abscissa; ○, dizygotic twins, the higher concentration of each pair plotted on the ordinate.

sera are shown in Fig. 2. There was no dependence of the absolute value of the difference of $\log_{10}$ IgE level on the interval between first and second samples ($b = -4.2 \times 10^{-4} \log_{10}$ IgE units per month ± 0.438 S.D., $t = 0.05$). One of the individuals studied had atopic asthma and had intermittent steroid and desensitization therapy prior to and during the 17.0 month interval between her first and second serum samples; the pair of IgE levels observed in the sera of this individual are identified in Fig. 2.

The paired serum IgE levels of monozygous and dizygous veteran twin sera are plotted in Fig. 3. The paired serum IgE levels of twin children are shown in Fig. 4.

Duplicate determinations of the IgE levels of 22 sera of unrelated individuals selected at random from the veteran twin sera were done in order to obtain an estimate of the variance (V_E) resulting from imperfect reproducibility inherent in the assay procedure. Duplicate assays of any single serum were done within the same run. The calculated values of the relevant statistical parameters are given in Table I. The variance resulting from random error (V_E) in the assay procedure is much less than the variance between paired sera in the other study groups and does not constitute a substantial factor in the following analysis. The mean intrapair variance for the IgE levels of the same individuals at different times (V_F) was only 3 per cent of the variance of the population from which the sample was drawn (V_{pF}) (which is equivalent to the mean intrapair variance expected of random pairings). Thus, the short-term fluctuations in the serum IgE level of a single individual relative to the range of population values is sufficiently small to allow analysis of genetic effects on IgE levels. The $\log_{10}$

TABLE I. Values of statistical parameters

Experimental group	No. of pairs (N)	Intrapair variance of $\log_{10}$ IgE (V)	Population variance of $\log_{10}$ IgE (V_p)	Mean of $\log_{10}$ IgE ($\bar{x}$)	Correlation coefficient and 95 % confidence interval
Same serum assayed twice	22	0.0007 (V_E)	0.299 (V_{pE})	1.603	$r' = 0.998$ $0.995 \leq r' \leq 0.999$
Monozygous twin veterans	54	0.0767 (V_{MZa})	0.2398 (V_{pMZa})	1.627	
Dizygous twin veterans	39	0.1872 (V_{DZa})	0.3530 (V_{pDZa})	1.715	
Monozygous twin children	10	0.017 (V_{MZb})	0.370 (V_{pMZb})	2.078	
Dizygous twin children	13	0.078 (V_{DZb})	0.243 (V_{pDZb})	1.848	
Same individual sampled twice $\Delta t = 17.2$ (range 6.5 to 63) months	23	0.0092 (V_F)	0.2559 (V_{pF})	1.827	$r = 0.948$ $0.879 \leq r \leq 0.978$
First sample			0.2776	1.839	
Second sample			0.2341	1.815	

IgE levels of first samples as a whole and of the group of second samples as a whole are quite similar (Table I); the difference of the mean and the ratio of variances are not significant ($t = 0.15$, $\nu = 44$; $F = 1.186$, $\nu = 22, 22$). The interval of storage is therefore unlikely to have affected the observed serum IgE level, assuming, as seems reasonable, that storage effects were not obscured by a secular trend.

The mean intrapair variance of monozygous veteran twins (V_{MZa}) was 8 times V_F , indicating that the cumulative differences between adult monozygous twins are substantially greater than might be expected on the basis of short-term fluctuations. However, the mean intrapair variance of the monozygous twin children (V_{MZb}), although larger than V_F , is not significantly so. Additionally, the mean intrapair variance of monozygous twin children is significantly less than that of the monozygous adult twins. These observations suggest that the differences in serum IgE levels between individuals with the same genotype arise largely after childhood.

Both the adult and child monozygous twins show significantly less mean intrapair variance of IgE levels than the mean intrapair variance of IgE levels of the corresponding groups of dizygous twins (Table II). This difference implies that there is a genetic effect on IgE levels. The mean intrapair variance of dizygous twins, both adults and children, is, in turn, less than the mean intrapair variance expected of random unrelated individuals drawn from the corresponding populations, indicating that the genetic similarity or environmental similarity, or both, between dizygous twins of a pair exert a significant effect on IgE levels.

The observed variances of the various populations studied are similar and no significant differences were found. However, the mean of the $\log_{10}$ IgE levels of the twin children, taken as a whole, is 1.948 ± 0.571 S.D., significantly different ($t = 2.252$, $p < 0.05$) from the mean $\log_{10}$ IgE levels of veteran twins taken as a whole (1.664 ± 0.535 S.D.). This difference may be attributed in part to errors in the IgE assay due to nonspecific blocking resulting in

TABLE II. Significance of ratios of variance

	Monozygous twin veterans N = 54 $V_{MZa} = 0.0767$	Dizygous twin veterans N = 39 $V_{DZa} = 0.1872$	Dizygous twin veterans population N = 39 $V_{pDZa} = 0.353$
Same individual sampled twice N = 23 $V_F = 0.0092$	F = 8.34 $\nu = 54, 23$ p < 0.001	F = 20.35 $\nu = 39, 23$ p < 0.001	
Monozygous twin veterans N = 54 $V_{MZa} = 0.0767$		F = 2.44 $\nu = 39, 54$ p < 0.005	
Dizygous twin veterans N = 39 $V_{DZa} = 0.1872$			F = 1.89 $\nu = 38, 39$ p < 0.05
Monozygous twin children N = 10 $V_{MZb} = 0.017$			
Dizygous twin children N = 13 $V_{DZb} = 0.078$			

increased IgE levels in the case of the twin children, for whom the volume of plasma available was insufficient to measure IgE levels, where appropriate, as the difference between absorbed and unabsorbed assay values. An additional possible source of the difference in mean IgE levels may have arisen due to the selection of the veterans as individuals suitable for induction into the armed services, which might exclude some individuals with high IgE levels and associated allergy. The mean $\log_{10}$ IgE level of the unselected population studied for temporal stability (1.827 ± 0.517) lies between the mean IgE level of the veterans and that of the children and is significantly different from neither ($t = 1.317$ and 0.755 , respectively).

Subsequent to the original observation by Johansson,³⁴ the association of elevated IgE levels with atopic disease has been studied extensively, and it is evident that atopic individuals often have serum IgE levels higher than those usually found in nonatopic individuals. The frequency of atopy as a function of IgE levels has not been explicitly addressed, presumably because the study would necessitate acquisition of serum IgE levels and allergic histories of a large number of unselected individuals. The available data may, however, be analyzed to show the frequency of atopy as a function of IgE levels if an assumption is made about the frequency of atopy in an unselected population. Table III contains a compilation of serum IgE levels measured by Spitz and associates⁶ in their study of atopic and nonatopic or normal individuals, and includes a calculation of the frequency of atopy for IgE levels within specified intervals, assuming a 16.7 per cent frequency of atopic disease in the unselected population.¹⁴ The calculated frequency of atopic disease will depend on the

Monozygous twin children N = 10 $V_{MZb} = 0.017$	Dizygous twin children N = 13 $V_{DZb} = 0.078$	Dizygous twin children population N = 13 $V_{pDZb} = 0.243$	Same serum twice N = 22 $V_E = 0.0007$
F = 1.85 $\nu = 10, 23$ p > 0.1	F = 8.48 $\nu = 13, 23$ p < 0.001		F = 13.14 $\nu = 23, 22$ p < 0.001
F = 4.51 $\nu = 54, 10$ p < 0.01			F = 109.57 $\nu = 54, 22$ p < 0.001
	F = 2.40 $\nu = 39, 13$ p < 0.05		F = 267.43 $\nu = 39, 22$ p < 0.001
	F = 4.59 $\nu = 13, 10$ p < 0.025		F = 24.29 $\nu = 10, 22$ p < 0.001
		F = 3.12 $\nu = 12, 13$ p < 0.05	F = 111.43 $\nu = 13, 22$ p < 0.001

TABLE III. Frequency of atopy as a function of IgE level

IgE (U./ml.)*	Number of individuals		Theoretical per cent atopic in unselected population† (c)
	Normal and nonatopic (a)	Atopic (b)	
0-19	26	5	4.5
20	12	4	7.6
21-63.5	13	4	7.1
64-100	11	3	6.8
101-150	10	12	22.9
151-350	13	13	19.8
351-700	11	23	34.1
> 700	0	14	~100.0
	$\Sigma a = 96$	$\Sigma b = 78$	

*From data of Spitz and associates,⁶ assuming 1 ng. was taken to be approximately one unit in the original data. (Spitz, E.: Personal communication.)

†Assuming 16.7 per cent atopic in the total unselected population:

$$\frac{\Sigma b}{K \Sigma a + \Sigma b} = 0.167; \quad K = \frac{(1 - 0.167) (78)}{(0.167) (96)} = 4.05;$$

$$\text{then } c = \frac{b}{4.05a + b} \times 100.$$

definition of atopy as well as the population studied, but it is evident that the observed frequency of atopic disease increases when serum IgE levels exceed 100 U. per milliliter and is very high at serum IgE levels of 700 U. per milliliter or more. Thus, individuals with high serum IgE levels are at a substantially greater risk of allergic disease and may therefore be less likely to be included in the veteran population.

The magnitude of genetic effects may be estimated by the function H. In the

case of the adult twins, $H = 0.592$ ($P \{0.324 \leq H \leq 0.749\} = 0.9$) and for the twin children, $H = 0.789$ ($P \{0.437 \leq H \leq 0.927\} = 0.9$). The observed H is larger for the twin children, consistent with the smaller mean intrapair variance of monozygous twin children previously noted. Although the genetic effects appear to be substantial, it is important to note that one pair of monozygous twins differed in IgE levels by a factor of 20 (1,223 and 60.7 U. per milliliter). The serum samples of this twin pair were found to be identical for the serum polymorphisms studied, which leaves little possibility of an error in zygosity determinations or in the identification of the sera. Additionally, changes as large as twofold in IgE levels with time were observed in some subjects; the extreme cases were a change from 16 to 34.2 U. per milliliter in 17.3 months and from 66 to 145 U. per milliliter in 19.2 months.

DISCUSSION

The data from the two groups of twins leave little doubt that, with respect to IgE levels, monozygous twins of a pair are substantially more similar to each other than are otherwise comparable dizygous twins of a pair. The extent to which these observations argue for hereditary effects ultimately depends on the validity of the assumption that monozygous twins of a pair are not significantly more similar to each other than are dizygous twins of a pair with respect to environmental factors relevant to the determination of IgE levels.

The data of Yunginger and Gleich²⁴ may be used to obtain an estimate of the variance resulting from short-term environmental differences. They studied the changes in IgE levels in a group of nonallergic individuals and another group containing only allergic individuals (not undergoing treatment) over the period from July to January, during which time the amount of airborne allergens changes markedly in the central United States, where their work was done. With few exceptions the serum IgE levels of allergic individuals were lower in January, but there was no significant change in the IgE levels of non-allergic individuals.

When analyzed by the methods used in this twin study (and assuming 2.4 ng. of IgE per unit), the mean intrapair variance in the allergic individuals from summer to winter was 0.0216 and the mean intrapair variance of the nonallergic individuals was 0.0068. This latter seasonal variation is similar to the variance resulting from random fluctuations (0.0092) in the individuals studied by us over a 17 month interval. Even in the case of allergic individuals, the seasonal effect is small relative to the differences in mean intrapair variances between the monozygous and dizygous veteran twins. Thus, it seems unlikely that differences of the magnitude observed between mean intrapair variances of monozygous and dizygous twins could have arisen as the result of presumably minor differences in the similarities in environment between twins of a pair, at least with the short-term effects (other than parasitic infestation³⁵) for which data are available.

There are no pertinent data with respect to the long-term environmental factors that may determine over-all IgE levels. Although it may reasonably be

assumed that a complete lack of exposure to antigenic stimulation will lead to virtual agammaglobulinemia,³⁶ it seems unlikely that differential antigenic exposure within realistic limits of common environmental variation within the United States would result in the large effects encountered in this study.

Variance due to nongenetic causes has usually been ascribed to environmental differences. This interpretation seems to be without substantial experimental support, and the nongenetic differences may as well be due largely to an accumulation of essentially random phenomena acting upon progenitor cells of potentially important clones or to other such random processes. Thus, the existence of monozygous twins discordant for allergy or having different IgE levels need not imply that manipulation of the environment (within the usual range of environmental variation) will materially affect the risk of developing high IgE levels. The increased mean intrapair variance of monozygous twin veterans relative to the monozygous twin children taken at face value would indicate that, over the long term, there is divergence of IgE levels in individuals with the same genotype. Whether this divergence is a result of indeterminacy in the IgE system or is a cumulative result of environmental effects cannot be decided on the basis of the data presented here.

Since the onset of atopic disease is commonly in childhood,¹⁵ the veteran population is likely to be lacking in individuals whose immediate-type hypersensitivities constituted a medical problem sufficiently severe to exclude them from military service, and thus the range of IgE levels would be to some extent truncated. Moreover, when twins are concordant for high IgE levels, there is increased probability of exclusion from the study group, since the exclusion of only one twin of a pair from military service results in the exclusion of both twins of a pair from the study. The parameter H may thus be expected to be to some extent underestimated, in the case of veteran twins, relative to the value that might be obtained in an unselected population. This possibility may also contribute to the observation of a smaller H for veterans than for children in this study.

The serum levels of IgG, IgA, and IgM in unselected adults are distributed much more closely about their mean values relative to the distribution of IgE levels; means and standard deviations computed as $\log_{10}$ values for the 30 adults studied by Buckley, Dees, and O'Fallon³⁷ are $\log_{10}$ IgG = 3.0257 mg. per 100 ml. $\pm$ 0.1117 S.D., $\log_{10}$ IgA = 2.4249 mg. per 100 ml. $\pm$ 0.2000 S.D., and $\log_{10}$ IgM = 1.8808 mg. per 100 ml. $\pm$ 0.1534 S.D. by comparison with the IgE values in the veterans studied by us, $\log_{10}$ IgE = 1.663 U. per milliliter $\pm$ 0.5428 S.D. Expressed as per cent of the mean, the standard deviations for IgG, IgA, and IgM are 3.7, 8.2, and 8.2 per cent, respectively, and for IgE, 32.9 per cent. Despite the relatively narrow distribution of the levels of IgG, IgA, and IgM, the heritability of the levels of these immunoglobulins individually in twin studies is high.^{38, 39} Correlations between levels of immunoglobulins G, A, M, and E have not been studied extensively; the limited data suggest that IgG and IgA levels are correlated in populations of normal individuals of a single race.^{40, 41} The genetic effects seen in twin studies are supported by the observa-

tions of elevated IgG levels in Negroes relative to the levels occurring in Caucasians.^{37, 42} The marked elevation of IgE levels, and to a lesser extent of IgG, IgA, and IgM levels as well, found in Filipinos by comparison with otherwise similar Caucasians⁴⁰ may additionally indicate a genetic effect on the levels of immunoglobulins in general.

Multigenic effects on IgE levels cannot be excluded. However, the multimodal distribution of IgE levels seen in many groups studied raises the possibility that IgE levels are determined by a small number of alleles at a single locus with a wide range of phenotypic expression for a given genotype; convincing examples of this exist with respect to other traits.^{43, 44} The possible overlap of phenotypes for a given genotype in middle-aged men is substantial; the extreme case of a 20 fold difference in IgE levels between monozygous twins is a formidable problem, for even if this difference is a result of extreme antigenic stimulation in the twin with the high serum IgE level, there is no simple way to identify similar occurrences. More encouraging for the possibility of fruitful pedigree analysis or analysis of siblings is the common limitation of differences in IgE levels in veteran monozygous twins to factors of 2 or 3 and the observation of good concordance in identical twin children.

The presence of IgE-mediated allergic disease may be contingent upon several factors among which the amount of IgE produced, reflected in the serum IgE level, appears to be of central, but not exclusive, importance. Among the other factors that have been studied are individual variation in the number of effector cells (mast cells)⁴⁵ and skin histamine content,⁴⁶ variation in the ability of effector cells (basophils) to be sensitized,^{47, 48} variation, possibly genetic, in the ability to produce antibody to specific locally prevalent allergens,⁴⁹⁻⁵¹ variation in the ability to be sensitized specifically by airborne allergens,⁵² and variation, possibly genetic, in the reactivity of target organs.^{17, 53, 54} A high serum IgE level is a major empirical risk factor, but whether the IgE level is directly affected by genetic factors or is secondarily determined by other factors that are genetically controlled has not been established.

Because of the individual variation in effector cell and end organ sensitivity, the inheritance of allergy may be expected to be substantially more complicated than the genetic determination of IgE levels. Differences in allergic manifestations between monozygous twins of a pair may not be associated with differences in serum IgE levels; two carefully studied pairs of monozygous twins discordant for allergic symptoms were concordant for serum IgE levels.⁵⁵ Penetrance on the order of 0.4 for allergy in monozygous twins has been calculated from an extensive twin study,⁵⁶ although this is likely to be an underestimate since a questionnaire method was used to gather data on both disease and zygosity. This demonstrated partial penetrance, and the evidence for several separate genetic factors is consistent with the failure to obtain convincing fits with either a multifactorial or a single locus with partial penetrance model¹⁴; it seems likely that there are indeed several genetic factors that may individually exhibit partial penetrance. At low serum IgE levels a multitude of factors may be relevant to the occurrence of allergic disease; at high serum IgE levels the serum IgE level

is an important risk factor. In the latter cases the genetic effect on IgE levels may be expected to correspond closely to the inheritance of allergy.

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REFERENCES

- 1 Ishizaka, K., Ishizaka, T., and Hornbrook, M. M.: Allergen-binding activity of γ E, γ G and γ A antibodies in sera from atopic patients; in vitro measurements of reaginic antibody, *J. Immunol.* **98**: 490, 1967.
- 2 Wide, L., Bennich, H., and Johansson, S. G. O.: Diagnosis of allergy by an in vitro test for allergen antibodies, *Lancet* **2**: 1105, 1967.
- 3 Johansson, S. G. O.: Serum ND levels in healthy children and adults, *Int. Arch. Allergy Appl. Immunol.* **34**: 1, 1968.
- 4 Gleich, G. J., Averbek, A. K., and Swedlund, H. A.: Measurement of IgE in normal and allergic serum by radioimmunoassay, *J. Lab. Clin. Med.* **77**: 690, 1971.
- 5 Johansson, S. G. O., Bennich, H., and Wide, L.: A new class of immunoglobulin in human serum, *Immunology* **14**: 265, 1968.
- 6 Spitz, E., Gelfand, E. W., Sheffer, A. L., and Austin, K. F.: Serum IgE in clinical immunology and allergy, *J. ALLERGY CLIN. IMMUNOL.* **49**: 337, 1972.
- 7 Arbesman, C. E., Ito, K., Wypych, J. I., and Wicher, K.: Measurement of serum IgE by a one-step single radial radiodiffusion method, *J. ALLERGY CLIN. IMMUNOL.* **49**: 72, 1972.
- 8 Bazaral, M., Orgel, H. A., and Hamburger, R. N.: IgE levels in normal infants and mothers and an inheritance hypothesis, *J. Immunol.* **107**: 794, 1971.
- 9 Oprea, W., Krause, H., and Stockberg, H.: Genetic aspects of serum IgE levels in atopic families, *Acta Allergol.* **27**: 247, 1972.
- 10 Bazaral, M., and Hamburger, R. N.: Genetic control of immunoglobulin E (IgE) levels in the human, *Clin. Res.* **20**: 258, 1972. (Abst.)
- 11 Hamburger, R. N., Orgel, H. A., and Bazaral, M.: Genetics of human serum IgE levels, in Goodfriend, L., Sehon, A. H., and Orange, R. P., editors: *Mechanisms in allergy: Reagin-mediated hypersensitivity*, New York, 1973, Marcel Dekker, Inc., p. 131.
- 12 Cooke, R. A., and VanderVeer, A.: Human sensitization, *J. Immunol.* **1**: 201, 1916.
- 13 Ratner, B., and Silberman, D. E.: Allergy—its distribution and the hereditary concept, *Ann. Allergy* **9**: 1, 1952.
- 14 VanArsdel, P. P., and Motulsky, A. G.: Frequency and heritability of asthma and allergic rhinitis in college students, *Acta Genet.* **9**: 101, 1959.
- 15 Weiner, A. S.: Zieve, J., and Fries, J. H.: The inheritance of allergic disease, *Ann. Eugen.* **7**: 141, 1936.
- 16 Adkinson, J.: The behavior of bronchial asthma as an inherited character, *Genetics* **5**: 363, 1920.
- 17 Tips, R. L.: A study of the inheritance of atopic hypersensitivity in man. *Am. J. Hum. Genet.* **6**: 328, 1954.
- 18 Osborne, R. H., and de George, F. V.: *Genetic basis of morphological variation*, Cambridge, Mass., 1959, Harvard University Press, pp. 15, 55.
- 19 Cavalli-Sforza, L. L., and Bodmer, W. F.: *The genetics of human populations*, San Francisco, 1971, W. H. Freeman & Company, p. 573.
- 20 Partanen, J., Brun, K., and Markkanen, T.: Inheritance of drinking behavior: A study on intelligence, personality and use of alcohol of adult twins, Helsinki, 1966, Finnish Foundation for Alcohol Studies, p. 55.

- 21 Kempthorne, O., and Osborne, R. H.: The interpretation of twin data, *Am. J. Hum. Genet.* 13: 320, 1961.
- 22 Rowe, D. S., Tackett, L., Bennich, H., Ishizaka, K., Johansson, S. G. O., and Andersson, S. G.: A research standard for human serum immunoglobulin E, *Bull. WHO* 43: 609, 1970.
- 23 Bazaral, M., and Hamburger, R. N.: Standardization and stability of immunoglobulin E (IgE), *J. ALLERGY CLIN. IMMUNOL.* 49: 189, 1972.
- 24 Yunginger, J. W., and Gleich, G. J.: Seasonal changes in serum and nasal IgE concentrations, *J. ALLERGY CLIN. IMMUNOL.* 51: 174, 1973.
- 25 Stokes, C. R., Hosking, C. S., Turner, M. W., and Johansson, S. G. O.: Urinary IgE: A reappraisal, *Eur. J. Immunol.* 3: 241, 1973.
- 26 Polmar, S. H., Waldmann, T., and Terry, W. D.: A comparison of three radioimmunoassay techniques for the measurement of serum IgE, *J. Immunol.* 110: 1253, 1973.
- 27 Jablon, S., Neel, J. V., Gershowitz, H., and Atkinson, G. F.: The NAS-NRC twin panel: Methods of construction of the panel, zygosity diagnosis, and proposed use, *Am. J. Hum. Genet.* 19: 133, 1967.
- 28 Feinlieb, M., Havlik, R. J., Kwiterovich, P. O., Tillotson, J., and Garrison, R. J.: The National Heart Institute twin study, *Acta Genet. Med. Gemellol.* 19: 243, 1970.
- 29 Berg, T., and Johansson, S. G. O.: Immunoglobulin levels during childhood, with special regard to IgE, *Acta Paediatr. Scand.* 58: 513, 1969.
- 30 Pearson, E. S., and Hartley, H. O., editors: *Biometrika tables for statisticians*, London, 1966, Cambridge University Press, vol. 1, p. 169.
- 31 Fisher, R. A., and Yates, F.: *Statistical tables for biological, agricultural and medical research*, Edinburgh, 1963, Oliver & Boyd, Ltd., p. 48.
- 32 Bailey, N. T. J.: *Statistical methods in biology*, New York, 1959, John Wiley & Sons, p. 91.
- 33 Fisher, R. A.: *Statistical methods for research workers*, Edinburgh, 1970, Oliver & Boyd, pp. 200, 217.
- 34 Johansson, S. G. O.: Raised levels of a new immunoglobulin class (IgND) in asthma, *Lancet* 2: 951, 1967.
- 35 Johansson, S. G. O., Mellbin, J., and Vahlquist, B.: Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND), *Lancet* 1: 1118, 1968.
- 36 Sell, S., and Fahey, J. L.: Relationship between γ -globulin metabolism and low serum γ -globulin in germ free mice, *J. Immunol.* 93: 81, 1964.
- 37 Buckley, R. H., Dees, S. C., and O'Fallon, W. M.: Serum immunoglobulins. I. Levels in normal children and in uncomplicated childhood allergy, *Pediatrics* 41: 600, 1968.
- 38 Allansmith, M., McClellan, B., and Butterworth, M.: The influence of heredity and environment on human immunoglobulin levels, *J. Immunol.* 102: 1504, 1969.
- 39 Kalf, M. W., and Hijmans, W.: Serum immunoglobulin levels in twins, *Clin. Exp. Immunol.* 5: 469, 1969.
- 40 Orgel, H. A., Lenoir, M. A., and Bazaral, M.: Serum IgG, IgA, IgM, and IgE levels and allergy in Filipino children in the United States, *J. ALLERGY CLIN. IMMUNOL.* 53: 213, 1974.
- 41 Lichtman, M. A., Vaughan, J. H., and Hames, C. G.: The distribution of serum immunoglobulins, anti- γ -G-globulin ("rheumatoid factors") and anti-nuclear antibodies in white and Negro subjects in Evans County, Georgia, *Arthritis Rheum.* 10: 204, 1967.
- 42 Lichtman, M. A., Hames, C. G., and McDonough, J. J.: Serum protein electrophoretic fractions among Negro and white subjects in Evans County, Georgia, *Am. J. Clin. Nutr.* 16: 492, 1965.
- 43 Harris, H.: Genetics of man: Enzyme polymorphisms in man, *Proc. R. Soc. Lond. (Biol.)* 164: 298, 1966.
- 44 Murphy, E., and Bolling, D.: Testing of single locus hypotheses where there is incomplete separation of the phenotypes, *Am. J. Hum. Genet.* 19: 322, 1967.
- 45 Hellstrom, B., and Holmgren, H. J.: Numerical distribution of mast cells in the human skin and heart, *Acta Anat.* 10: 81, 1950.
- 46 Zachariae, H.: Histamine in human skin: A spectrofluorometric assay, *Acta Derm. Venereol.* 44: 219, 1964.
- 47 Levy, D. A., and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen, *J. Immunol.* 97: 203, 1966.
- 48 Ishizaka, T., Soto, C. S., and Ishizaka, K.: Mechanisms of passive sensitization. III.

- Number of IgE molecules and their receptor sites on human basophil granulocytes, *J. Immunol.* 111: 500, 1973.
- 49 Kisil, F. T., Gyenes, L., and Schon, A. H.: The heterogeneity of allergen-antibody systems related to ragweed allergy in man, *J. ALLERGY* 47: 1, 1971.
 - 50 Levine, B. B., Stember, R. H., and Fotino, M.: Ragweed hay fever: Genetic control and linkage to HL-A haplotypes, *Science* 178: 1201, 1972.
 - 51 Marsh, D. G., Bias, W. B., and Hsu, S. H.: Association of the HL-A7 crossreacting group with a specific reaginic antibody response in allergic man, *Science* 179: 691, 1973.
 - 52 Salvaggio, J. E., Cavanaugh, J. J. A., Lowell, F. C., and Leskowitz, S.: A comparison of the immunologic responses of normal and atopic individuals to intranasally administered antigen, *J. ALLERGY* 35: 62, 1964.
 - 53 Felarca, A. B., and Itkin, I. H.: Studies with quantitative-inhalation challenge technique. I. Curve of dose response to acetyl-beta-methylcholine in patients with asthma of known and unknown origin, hay fever subjects, and nonatopic volunteers, *J. ALLERGY* 37: 223, 1966.
 - 54 Szentivanyi, A.: The beta adrenergic theory of the atopic abnormality in bronchial asthma, *J. ALLERGY* 42: 203, 1968.
 - 55 Falliers, C. J., Cardoso, R. R. de A., Bane, H. N., Coffey, R., and Middleton, E., Jr.: Discordant allergic manifestations in monozygotic twins: Genetic identity versus clinical, physiologic, and biochemical differences, *J. ALLERGY* 47: 207, 1971.
 - 56 Edfors-Lubs, M. L.: Allergy in 7000 twin pairs, *Acta Allergol.* 26: 249, 1971.
 - 57 Neel, J. V., and Schull, W. J.: Human heredity, Chicago, 1954, University of Chicago Press, p. 274.
 - 58 Rowe, D. S., Boyle, J. A., and Buchanan, W. W.: Plasma immunoglobulin concentrations in twins, *Clin. Exp. Immunol.* 3: 233, 1968.

APPENDIX

Computation of intrapair variance in twin studies

The average intrapair variance may be computed for dizygous twins as¹⁸

$$V_{Dz} = \frac{\sum (x_i - x_i')^2}{2N}, \quad (1)$$

where x_i and x_i' are the observed values for the i th pair of dizygous twins, regardless of which twin of the i th pair is designated x_i or x_i' . This approach is implicit in the analysis by Neel and Schull⁵⁷ and was used in conjunction with the analysis of variance by Osborne and de George.¹⁸ Subsequently, this or similar methodology has been used in the analysis of immunologic data,^{38, 39, 58} as well as in other applications.

The occurrence of V_{Dz} significantly less than the variance expected from random pairings of individuals from the same population indicates that genetic or environmental effects have resulted in greater similarity between dizygous twins of a pair than would occur randomly. A method of estimating the intrapair variance (V_p) expected of random pairings of individuals in a population may be derived from the equation above:

$$V = \frac{\sum (x_k - x_k')^2}{2N}, \quad (2)$$

where x_k and x_k' are the k th of N samples drawn, randomly and independently of each other, from the same population of unrelated individuals. Then,

$$V (2N) = \sum (x_k)^2 - \sum x_k x_k' + \sum (x_k')^2 - \sum x_k x_k'; \quad (3)$$

if x_k and x_k' are independent,

$$V (2N) = \sum (x_k)^2 - \bar{x}_k \sum x_k' + \sum (x_k')^2 - \bar{x}_k' \sum x_k \quad (4)$$

since $\mu_x = \mu_{x'}$, $\bar{x} \approx \bar{x}'$, and since $\sigma_x^2 = \sigma_{x'}^2$, $s_x^2 \approx s_{x'}^2$

$$V \approx \frac{\sum (x)^2 - (\sum x)^2/N}{N}. \quad (5)$$

V , for large N , approximates the sample variance (s^2) of the population from which x_k and x_k' are drawn.

The mean variance of random pairs of dizygous twins may be computed using equation 5, with the x_k taken to be one randomly chosen member of a twin pair. The available data may

be more fully utilized if V_p' is calculated as well for the other member of the twin pairs and the two variances averaged,

$$V_{DZ} = \frac{\sum x_i^2 - (\sum x_i)^2/N}{2N} + \frac{\sum x_i'^2 - (\sum x_i')^2/N}{2N}, \quad (6)$$

where x_i and x_i' are the values of members of the i th of N twin pairs and a member of a twin pair is designated as x_i or x_i' by a random process. The degrees of freedom associated with V_p in Equation 6 is a function of the correlation between x and x' , but has a lower limit of $N - 1$ than may conveniently be used. This method of calculation of the intrapair variance is otherwise independent of the degree of correlation between twins of a pair.

The method expected of random pairings used by Osborne and de George¹⁸ and others^{39, 58} is

$$V_{ip} = \frac{2 \left(\sum [(x_i + x_i')/2]^2 - \frac{[\sum (x_i + x_i')/2]^2}{N} \right)}{N - 1}, \quad (7)$$

where x_i and x_i' are the values of the i th of N pairs of dizygous twins.

This equation may be expanded:

$$2(N - 1) V_{ip} = [\sum (x_i)^2 - (\sum x_i)^2/N] + [\sum (x_i')^2 - (\sum x_i')^2/N] + 2[\sum x_i x_i' - (\sum x_i \sum x_i')/N] \quad (8)$$

and if x_i and x_i' are independent,

$$\sum x_i x_i' = (\sum x_i \sum x_i')/N - 1, \quad (9)$$

then $V_{ip} = s^2$, where s^2 is the sample variance of the population from which x_i and x_i' are drawn.

However, if the values x and x' of the members of the twin pairs are correlated, V_{ip} is larger than s^2 ; in the extreme case where $x_i = x_i'$, $V_{ip} = 2s^2$.

In many cases of interest, the values for twins of a dizygous pair are indeed correlated and in those cases, Equation 8 will result in an overestimate of the significance of the ratio between V_{DZ} and the variance expected to result from random pairings of unrelated individuals.

Mean intrapair variances have also been calculated^{19, 58} as

$$V' = \sum (x_i - x_i')^2/N, \quad (10)$$

which results in twice the calculated value of the variance relative to the value that would be obtained if Equation 1 were used. Equation 10 is equivalent for most purposes to Equation 1 since, in estimating H or in tests of significance, constants in the denominator cancel out. However, variance expected to result from random pairing of samples from the dizygous twin population, if calculated as in Equation 7, will be an appropriate estimate (relative to Equation 10) of the variance only if $x_i = x_i'$; if x_i is independent of x_i' , the calculation will yield a V_{ip} , which is only half of the variance expected of random samples. In one case,⁵⁸ this problem apparently contributed to the rejection of the hypothesis that there is a significant genetic effect on immunoglobulin levels in adults.