The NAS-NRC Twin Panel: Methods of Construction of the Panel, Zygosity Diagnosis, and Proposed Use

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INTRODUCTION

DESPITE THE MANY problems inherent in the twin method, it remains an approach of great potential value in providing clues as to the genetic factor in the etiology of a wide variety of traits. With few exceptions, such as the recent studies of Kallmann and Sander (1949 *et seq.*), Cederlöf *et al.* (1961), and Harvald and Hauge (1965), the larger-scale twin studies of the past or present have been concerned with children or young adults. This reflects the greater mobility and lesser tractability of older twins. Consequently, the potential contributions of the twin method in determining the role of familial and genetic factors in a wide variety of diseases of middle and old age remain largely unrealized.

This paper describes (1) an effort to establish a large panel of adult twins through the facilities of the Veterans Administration (VA), (2) an exploration of the feasibility of determining, with a known probability of error, the zygosity of each pair, through data on file with Federal agencies or obtainable by a simple questionnaire, and (3) an exploration of the means whereby, once the panel is set up, an ongoing system of information retrieval concerning the twins can be established. Finally, the problems inherent in maintaining this panel as a genetic resource will be considered.

It is immediately obvious that the panel to be described is neither representative nor cross-sectional. It is restricted to twins both of whom survived to military age and both of whom were then found physically fit for military service. Thus, twin pairs wherein one member had a serious congenital defect or childhood disease with lasting major sequelae are not represented, and pairs wherein one or both members have experienced the early onset of such chronic diseases as diabetes mellitus or essential hypertension are not

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represented. Nevertheless, there are enough diseases whose usual onset is subsequent to the thirties (such as the malignancies, cardiovascular disease, and the arthridides) to make it clear that a panel such as this can yield much useful information. We shall return in the discussion to a consideration of the limitations on the panel.

THE POTENTIALITIES OF THE VA FOR TWIN STUDIES; THE EFFORT TO LOCATE VETERAN TWINS

The special value of the veteran population for medical follow-up studies has been demonstrated by the experience of the Follow-up Agency, National Academy of Sciences–National Research Council (NAS–NRC). The records of the armed services and of the VA provide nearly complete information as to mortality of veterans and substantial, although not complete, information on morbidity. In addition, the size of the veteran population permits the creation of large rosters of men with even relatively rare diseases. The various VA benefits available to veterans also make it possible to locate a large proportion of any group of living persons selected.

In 1955, experiments were initiated to explore methods of identifying twins who served in the Armed Forces during World War II. The method settled on was to obtain from the various state and city vital statistics offices in the U.S. copies of the birth records of all white male twins born in the years 1917–1927 and to match the names thus obtained against the VA Master Index (VAMI) to determine which twins survived with both entering military service. About 99% of all World War II veterans are represented in VAMI.

Co-operation of 42 vital statistics offices was obtained (all of the continental U. S. except Arizona, Connecticut, Delaware, Georgia, Maine, Missouri, New Orleans, Utah, and Vermont). Over 54,000 eligible pairs were found by the participating offices, and, of these, 16,000 pairs were identified by the VA as both having served in the armed services. For 15,000 pairs, one member only was identified as a veteran, and for 23,000 pairs neither was identified. Thus, 108,000 names were searched against the VAMI, of which 47,000, or 43.5% were matched.

It is not possible to tell just why the proportion of matches was so low. For a white male cohort born in 1920, about 86% survived to 1942. About 80% of the survivors served in the military forces in World War II, so that we might have expected to match about 69% rather than 43.5%. Possible reasons for the discrepancy include higher mortality in the twins than in singletons born in the same year, higher rates of rejection for physical disability, and failures to match correctly at VAMI because of changes in name or inaccurate birth dates shown on the VAMI index card. It must be realized that the VAMI file is ordinarily searched only when a military identifying number is known, thus assuring correct identification. The file clerks, when searching for twins, had to rely on name and date of birth, and there probably were many failures to match men for whom cards were in fact on file.

There was a striking tendency to match either both or neither of the twins at VAMI. Given that one member of a pair was matched, the probability of

matching his twin was nearly 52% in contrast to only 39% if the index member was not matched. This may indicate a greater diligence by the clerks when searching for the twin to an already matched man, and perhaps the converse.

ESTABLISHMENT OF A PANEL OF TWINS OF KNOWN ZYGOSITY

Under ideal circumstances, the determination of zygosity for each twin pair would be based on a personal contact with each twin, in the course of which all the data on morphological characteristics and genetic traits thought necessary to an accurate diagnosis of zygosity would be obtained. Such an extensive undertaking was clearly not feasible during these preliminary studies. An alternative was to explore the reliability with which diagnosis could be established on the basis of material obtainable either from Federal Government files or by correspondence with the twins. The pursuit of this alternative required that there be created a subpanel of twins in which zygosity had been determined with a high measure of accuracy.

Selection of Twins for Inclusion in Subpanel

A list of 298 twin pairs was drawn up, the sole criteria for inclusion in this list being (1) both members of pair alive, with last known address for both members in the lower peninsula of the State of Michigan or upstate Illinois, and (2) availability of fingerprints from the Federal Bureau of Investigation. These 298 pairs were *all* the pairs in these two geographical areas meeting these criteria at this time in the study (1960). A brief letter was sent to each twin, informing him that a study of twin veterans was to be undertaken, asking his co-operation, and promising him, at the completion of the study, a report on his blood types and our opinion as to whether he was an identical or fraternal twin.

Seven medical students then undertook, during a summer vacation, to establish contact with these 596 persons and enlist their co-operation in the investigation.* A total of 257 pairs were finally included in the study. Of the remainder, in 23 instances one or both twins either could not be traced or no longer resided in the geographical area under consideration; in 15 instances one or both members of the pair declined participation in the study; in one instance one member of the pair had died recently; in one instance the pair were not twins;† and in one instance the twin pair was Negroid and excluded from the study on this basis.

Tests Used in Establishing Zygosity

Each twin included in the investigation was classified as to eye and hair

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†This fact was subsequently independently uncovered in the standard processing of the twin records, so that it cannot be taken as an indication of the degree to which nontwins might be inadvertently included in the panel.

TABLE 1. PHENOTYPES DETERMINED FROM BLOOD AND SALIVA SPECIMENS OF 257 SINGLE MEMBERS OF WHITE VETERAN TWIN PAIRS RESIDING IN MICHIGAN AND ILLINOIS

System	Phenotype	Number	Frequency	System	Phenotype	Number	Frequency
ABO	0	98	.381	Rh	CDe	47	.183
	A_1	76	.296		CcDe	84	.327
	A_2	33	.128		CcDEe	32	.125
	B	34	.132		cDE	4	.016
	A_1B	11	.043		cDEe	31	.121
	A_2B	5	.019		cDe	5	.019
		257	.999		cde	43	.167
					cdEe	3	.012
MNSs	MS	23	.089		CDEe	1	.004
	MSs	46	.179		C^wDe	4	.016
	Ms	19	.074		C^wcDe	1	.004
	MNS	8	.031		CcD^ue	1	.004
	MNSs	52	.202		cD^uEe	1	.004
	MNs	53	.206			257	1.002
	NS	2	.008				
	NSs	8	.031				
	Ns	46	.179	P	P_1	202	.786
		257	.999		P_2	55	.214
						257	1.000
Fy	Fy(a+)	179	.696				
	Fy(a−)	78	.304	Hp	Hp(1-1)	37	.146
		257	1.000		Hp(2-1)	125	.492
					Hp(2-2)	92	.362
K	K	1	.004			254	1.000
	Kk	15	.058				
	k	241	.938				
		257	1.000				
Sec	Sec	189	.741				
	Nonsec	66	.259				
		255	1.000				
Gm	Gm(1)	112	.441				
	Gm(−1)	142	.559				
		254	1.000				

color and hair pattern (baldness). Frontal and three-fourths profile photographs were taken, and blood, saliva, and hair samples obtained. A brief educational and occupational history was taken (cf. Neel, 1962).

Each blood specimen was typed at the Department of Human Genetics, University of Michigan, with reference to eight genetic systems (see Table 1). In addition, the saliva specimen was typed with reference to the ABH-secretor trait. Whenever twins were found to differ in only one or two laboratory tests, this finding was checked, either on the basis of a stored frozen specimen of blood, if available, or on the basis of a repeat specimen. Unfortunately, at the time the field work was discontinued, there were 19 pairs for which

TABLE 2. GENE FREQUENCIES DETERMINED FROM BLOOD AND SALIVA SPECIMENS OF 257 SINGLE MEMBERS OF WHITE VETERAN TWIN PAIRS RESIDING IN MICHIGAN AND ILLINOIS

System	Gene	Frequency	System	Gene	Frequency
ABO	*O*	0.620	P*	P_1	0.537
	A_1	0.184		P_2	0.463
	A_2	0.097			
	B	0.099	Duffy*	Fy^a	0.449
				Fy^b	0.551
MNSs	*MS*	0.289			
	Ms	0.274	Kell	*K*	0.032
	NS	0.046		*k*	0.968
	Ns	0.392			
			Secretor	*Se*	0.491
Rh	*CDe*	0.413		*se*	0.509
	cDE	0.124			
	cDe	0.022	Gm*	Gm^1	0.252
	cde	0.403		Gm^5	0.748
	cdE	0.014			
	CDE	0.005	Hp	Hp^1	0.392
	C^wDe	0.010		Hp^2	0.608
	CD^ue	0.005			
	cD^uE	0.005			

*Only one reagent was used in testing. Calculations are based on a two-allele system.

some item of information was lacking. There was thus left 238 pairs of twins.

The observed distribution of the blood types, *based on only one member of each of the 257 pairs,* is given in Table 1, and the gene frequencies (calculated by the methods described by Mourant, 1954) in Table 2. Using the approach described by Sutton *et al.* (1955) and Maynard Smith and Penrose (1955) and the gene frequencies derived from the twins themselves, we calculate the mean probability of monozygosity for twins concordant with respect to all nine traits to be .970. We will return later to the possibility that population heterogeneity may raise problems for this calculation.

Twins with confirmed serological differences were classified as dizygotic. Then the morphological information available for each of the remaining pairs was scrutinized with reference to differences in facial features (ear shape, nose shape, hairline, lip structure, etc.) which would establish dizygosity. The final assignment of zygosity was as follows: (1) discordant serologically, dizygotic—122 pairs; (2) concordant serologically but with significant morphological differences, probably dizygotic—seven pairs; (3) concordant serologically and morphologically—103 pairs; and (4) unassigned—25 pairs. With respect to the unassigned pairs, in three instances there were minimal morphological differences, confirmed on inspection by two of us (H. G. and J. V. N.), which could not be ignored but seemed an inadequate basis for a diagnosis of dizygosity; in one instance, there were apparent differences on the photographs which, again, seemed inadequate for a diagnosis of dizygosity;

in two instances, there were equivocal serological differences which are probably valid but could not be checked with a fresh specimen; and, as noted above, in 19 pairs some item of information was missing. Because the primary purpose of establishing this panel was to provide material of maximum validity, it was felt that the conservative course was not to force the diagnosis. Accordingly, there resulted from this approach 232 twin pairs for whom the diagnosis of zygosity seems reasonably sure. The proportion of the concordant pairs who were thought to be dizygotic (7/110, or 6.4%) is in satisfactory agreement with expectation on the basis of the serological tests performed and gene frequencies in the group. These 232 pairs constitute a panel that could be used in an effort to develop an approach that would maximize the probability of an accurate diagnosis of zygosity from material already on file concerning the twins.

In addition to the twin panel just described, a second resource was available with which to explore the diagnosis of twin zygosity from such material as would be available from veterans' records. Sutton *et al.* (1962) have described a study of a panel of 82 like-sexed twin pairs, of high school and college age, also carried out at the University of Michigan. Although fingerprints were obtained, the diagnosis of zygosity was based on serological and morphological evidence. After the exclusion of one pair because of technically unsatisfactory fingerprints, there was available a sample composed of 30 pairs discordant serologically, dizygotic; seven pairs concordant serologically but with significant morphological differences, probably dizygotic; and 44 pairs concordant serologically and with no significant morphological differences, considered monozygotic. With the battery of diagnostic tests employed in this study (five blood types and the secretor trait), the mean probability of monozygosity for concordant twins was 0.90. Thus, of the 51 sets of concordant twins among the total of 81 studied, 5.1 pairs were expected to be dizygotic; seven pairs were so classified—again satisfactory agreement. However, in both this panel and our own, the departure from expectation involves an excess of serologically concordant dizygotic pairs, raising the possibility that small morphological differences are unduly influencing the decision or that, because of preferential mating within ethnic groups with differing gene frequencies, there is an excess of concordant dizygotic pairs over expectation based on the assumption of random mating, an extension of the Wahlund principle.

A DISCRIMINANT FUNCTION FOR DIAGNOSIS OF ZYGOSITY FROM MATERIAL ON FILE IN FEDERAL AGENCIES

At the time of entry of a person into the Army or Navy, information is recorded as to his height, weight, eye color, hair color, complexion, and body build. Height and weight are recorded in inches and pounds, but the other items are characterized qualitatively and in a nonstandardized way. Therefore, while the anthropometric measures might play a supportive role in a method of zygosity determination, it was necessary to find some more solid foundation.

TABLE 3. STATUS OF SEARCH FOR FINGERPRINTS AT FBI AS OF 31 MARCH 1965

Number of pairs sent	5,452	
Prints found for both members		4,091
Prints found for one member only		1,136
Prints found for neither member		225
Yield for *pairs:*	4,091/5,452 =75.0%	
Yield for *individuals:*	9,318/10,904=85.5%	

Early in World War II it became general practice to send the fingerprints of recruits to the Federal Bureau of Investigation (FBI). Various workers (Maynard Smith and Penrose, 1955; Nixon, 1956; Slater, 1963; Slater *et al.*, 1964; and others) have indicated that fingerprints may be quite useful in the diagnosis of zygosity, and it was hoped that the information in the FBI files might serve as the foundation required.

Through the great courtesy of the FBI, microfilm copies of the fingerprint cards were obtained for a large number of the twins. Because of the press of routine work at the FBI, the task of searching files had to be done at odd moments and at this writing is not yet complete. However, a total of 5,452 pairs had been searched with results as shown in Table 3. The methods of diagnosis reported here have been worked out on this portion of the roster.

It was not feasible to count ridges for tens of thousands of digits, but the FBI classification (Federal Bureau of Investigation, 1963) was available for each digit. This consists of the *pattern,* classified as arch, tented arch, ulnar loop, radial loop, inner whorl, outer whorl, and the *ridge count* which is provided explicitly only for loops. Because, by definition, the ridge count for an arch or tented arch pattern is zero, these counts were present implicitly.

The task was to devise a method for using the already coded fingerprint classifications in the diagnosis of zygosity. There are 28 different possible combinations of patterns for the two corresponding digits for a pair of twins. If data were available for M pairs of twins known to be monozygotic and D pairs known to be dizygotic and if we denote by $M(i,j|f)$ and $D(i,j|f)$ (with $i \leq j$) the number of pairs of each kind for which the pattern combination i,j is found on digit f ($f = 1, \ldots, 10$), then the ratio

$$R(i,j|f) = \frac{M(i,j|f)/M}{D(i,j|f)/D}$$

would be an estimate of the relative odds in favor of the diagnosis "monozygotic," given that a pair had the combination of patterns i and j on the f^{th} digit. If combinations on different digits were statistically independent, then the combined odds ratio for all ten digits could be obtained by multiplying together the odds ratios for the individual digits.

Thus far nothing has been said about the ridge counts, which are available for pattern types 3 and 4, radial and ulnar loops. If we denote by X a particular ridge count (an integer ranging from 1 to about 30), then the conditional odds ratio, for a particular digit, f, and particular pattern combination, i,j, of which just one is a 3 or 4, could be estimated as:

$$R(X|i,j,f) = \frac{M(X,i,j|f)/M(i,j|f)}{D(X,i,j|f)/D(i,j|f)}$$

where we denote by $M(X,i,j|f)$ the number of monozygotic pairs having on digit f the pattern combination i,j and having a ridge count of X on the loop. By multiplying these ridge-count odds ratios together with the pattern odds ratios previously defined, information of both kinds could be combined.

Finally, if both i and j were, say, 4 (ulnar loops), thus making two ridge counts available, it would seem natural to consider the difference of the two ridge counts, say d, obtaining:

$$R(d|4,4,f) = \frac{M(d,4,4|f)/M(4,4|f)}{D(d,4,4|f)/D(4,4|f)}$$

The great difficulty in the procedure outlined above is the requirement for a very large sample of twins of known zygosity in order to permit reasonably reliable estimates of the many ratios. Clearly the sample of pairs diagnosed serologically would not nearly suffice. It was necessary, therefore, to use a stratagem which might lead to a scoring system of diagnostic usefulness and which could, at any rate, be tested against known results. The "anthropometric measurements" (height, weight, eye color, and hair color) were available, so it was decided first to devise a scoring system for zygosity based on these observations alone. The anthropometric scores (A scores) were then used to divide the pairs into three groups: those most similar, those least similar, and the intermediate group. It was expected that the group least similar as measured by the A score would contain relatively few monozygotic pairs, while the group most similar would contain a majority of monozygotic pairs, perhaps even a large majority. The extreme groups defined by the A score were used in place of the groups known to be MZ and DZ, admittedly at the cost of some precision but with the assurance of having some means at the end of testing the result.

The sample was restricted to the 2,805 pairs for which the data on the two members were recorded not more than a year apart (otherwise even large weight differences between the members of a pair would be uninformative). Even the devising of an anthropometric score had to be accomplished by a "boot strap" operation; that is, two groups were designated arbitrarily as "alike" and "not alike" on height and weight, and from these it was determined which eye-color and hair-color combinations should be considered "alike" and "not alike." This done, the newly defined hair- and eye-color combinations were used as criteria by which to refine the height-difference and weight-difference groupings. These alternations were continued until no further changes occurred.

We may summarize the results by saying that, on the basis of the four anthropometric observations, the 2,805 pairs were divided into three groups:

Group	All pairs		Pairs diagnosed serologically			
			Monozygotic		Dizygotic	
	Number	Per cent	Number	Per cent	Number	Per cent
1	658	23.5	30	39.0	11	11.2
2	1,515	54.0	43	55.8	43	43.9
3	632	22.5	4	5.2	44	44.9
Total	2,805	100.0	77	100.0	98	100.0

Groups 1 and 3 of 658 and 632 pairs were chosen as the criterion groups for the development of the fingerprint scoring system. As it turned out, where the serological diagnoses became available for the Michigan-Illinois sample discussed above, in Group 1, 30/41 or 73% were MZ, while in Group 3, 44/48 or 92% were DZ.

Pattern Scores

Tables were constructed for each digit, showing the number of pairs with each combination of patterns. These are exemplified in Table 4 for the right thumb. By way of examples for right thumbs, meeting whorls were found in both members in 13/658 of Group 1 (similar) pairs but in only 2/632 of Group 3 (dissimilar) pairs, for an odds ratio of 6.2 to 1. For the combination ulnar loop with inner whorl the proportions were, respectively, 12/658 and 21/632, so the odds ratio for this combination is 0.55 to 1.

The logarithms of the observed odds (or likelihood) ratios were used as scores, so that scores for different digits could later be combined by addition:

$$S(i,j|f) = 100\{2 + \log [M(i,j|f) + \tfrac{1}{2}] - \log [D(i,j|f) + \tfrac{1}{2}] - \log M + \log D\}$$

The number 2 was added to the sum of the logarithms to make it positive and the factor 100 to eliminate decimals. The quantity ½ was added to the observed number of pairs before taking the logarithm to prevent the logarithm from becoming infinite. For a justification of this particular quantity, see Appendix 1. In some instances of rare combinations, as in the arch-arch

TABLE 4. NUMBER OF PATTERN COMBINATIONS, RIGHT THUMB, FOR GROUP 1 (SIMILAR) AND GROUP 3 (DISSIMILAR) PAIRS.
GROUP 1 COUNT IS SHOWN AS NUMERATOR, GROUP 3 COUNT AS DENOMINATOR.

	Total, digits	Pattern type							
		Arch	Tented arch	Radial loop	Ulnar loop	Inner whorl	Outer whorl	Meeting whorl	Unspecified whorl
Total, digits	1,316/1,264	14/27	2/1	4/2	756/708	54/56	398/404	86/63	1/3
Arch	14/27	3/3	0/1	0/0	6/15	0/0	2/5	0/0	0/0
Tented arch	2/1		0/0	0/0	2/0	0/0	0/0	0/0	0/0
Radial loop	4/2			0/0	2/1	0/1	2/0	0/0	0/0
Ulnar loop	756/708				314/246	12/21	93/159	12/20	0/0
Inner whorl	54/56					3/6	23/19	13/3	0/0
Outer whorl	398/404						121/91	35/36	1/3
Meeting whorl	86/63							13/2	0/0
Unspecified whorl	1/3								0/0
Unknown	1/0				1/0				

TABLE 5. PATTERN SCORES

Combination of patterns*	Digit									
	Thumb		Index		Middle		Ring		Little	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
12 and any	200	200	200	200	200	200	200	200	200	200
1 and 1	213	213	227	227	226	226	212	212	228	228
2 and 2	200	200	221	221	225	225	235	235	235	235
3 and 3	222	222	208	204	248	248	222	222	200	200
4 and 4	211	207	217	214	206	203	211	207	204	204
5 and 5	204	222	224	207	240	234	239	218	204	225
6 and 6	212	204	207	221	234	240	209	239	245	204
7 and 7	273	273	216	216	261	261	244	244	253	253
1 and 2	206	206	203	203	200	200	253	253	253	253
1 and 3	200	200	152	152	178	178	200	200	200	200
1 and 4	170	170	187	166	171	174	158	158	104	104
1 and 5	148	148	172	64	116	116	116	116	105	105
1 and 6	148	148	64	172	116	116	116	116	105	105
1 and 7	148	148	64	64	116	116	116	116	105	105
1 and 8	148	148	141	141	116	116	116	116	105	105
2 and 3	200	200	162	199	200	200	200	200	200	200
2 and 4	200	200	212	193	205	198	200	177	196	196
2 and 5	64	64	156	156	141	141	89	89	157	157
2 and 6	64	64	156	156	141	141	89	89	157	157
2 and 7	64	64	156	156	141	141	89	89	157	157
2 and 8	64	64	156	156	141	141	89	89	157	157
3 and 4	200	200	200	202	202	202	212	212	200	200
3 and 5	200	200	202	194	205	205	181	181	200	200
3 and 6	200	200	173	213	205	205	181	181	200	200
3 and 7	200	200	173	194	205	205	181	181	200	200
3 and 8	200	200	173	194	205	205	181	181	200	200
4 and 5	177	181	168	203	172	188	161	188	175	181
4 and 6	177	200	200	173	173	224	194	152	178	144
4 and 7	177	200	200	203	216	184	165	152	175	144
4 and 8	177	183	183	186	183	192	185	187	179	181
5 and 6	208	195	186	189	196	225	180	188	200	200
5 and 7	259	212	226	192	205	206	200	203	200	200
6 and 7	199	248	233	250	196	222	207	185	200	200
8 and 5, 6, 7, or 8	212	218	216	216	223	225	207	213	237	228

*1, arch; 2, tented arch; 3, radial loop; 4, ulnar loop; 5, inner whorl; 6, outer whorl; 7, meeting whorl; 8, undetermined type whorl; 12, unknown.

combinations on the thumb, data for the left and right thumbs were combined to obtain a common score. The final pattern scores are shown in Table 5.

Certain general points are apparent in Table 5. Patterns of the same type on the homologous digits of a pair of twins always have a pattern score of 200 or more. However, the strength of the evidence varies considerably, the maximum being 273 (odds ratio of about 5 to 1) for meeting whorls on both left or both right thumbs. Combinations of arches with tented arches are also in favor of monozygosity but give substantial evidence only for the ring and little fingers. Arches tend *not* to be associated with loops, either

radial or ulnar, and are seldom found with whorls in the pairs of "similar" twins, i.e., those in Group 1. Tented arches appear commonly with loops but are infrequently found in association with whorls. Generally, whorls of all types are found in association.

Ridge-Count Scores

There are three possible situations with regard to ridge counts on a pair of digits: Both may be loops and have ridge counts, only one may have a count, or neither may. In the last instance, we assign a ridge-count score of 200 (which denotes no information). The kinds of information used in the first two instances are illustrated in Tables 6 and 7. From Table 6, it may be seen that the ulnar loop ridge counts in Group 1 are on the average somewhat larger than in Group 3; that is, among similar twins, an ulnar loop on the right thumb, when accompanied by an outer whorl on the homologous digit, is more likely to have a high ridge count than is true among dissimilar twins. Similarly, from Table 7 it is plain that when the thumbs of both members of a pair are ulnar loops, the differences of the ridge counts are smaller among similar than among dissimilar pairs.

Study of the logarithms of the ratios $R(X|i,j,f)$ showed that they were at least approximately linear functions of X, as might have been expected.* The score for each ridge count or ridge-count difference was then calculated using linear regression functions fitted to the values:

$$S(X|i,j,f) = 100\{2 + \log [M(X,i,j|f) + \tfrac{1}{2}] - \log [D(X,i,j|f) + \tfrac{1}{2}] - \log [M(i,j|f) + \tfrac{1}{2}] + \log [D(i,j|f) + \tfrac{1}{2}]\}$$

The method of fitting the regression lines is described in Appendix 2 and the parameters of the regression lines obtained are shown in Tables 8 and 9.

In Table 8, a negative sign for the coefficient of the loop ridge count indicates that in similar pairs the ridge count tends to be small, whereas a positive sign shows that the count tends to be high. Thus, among similar pairs, loop counts tend to be low when associated with arches or tented arches on the homologous digit of the sibling and high when associated with inner or outer whorls. Similarly it is apparent, from the fact that all the coefficients in Table 9 are negative, that the differences of ridge counts are smaller among similar pairs than among dissimilar pairs.

The next task was to use the fingerprint F score (average of all ten digits) to refine the scoring system for the anthropometric variables. After restriction

*Suppose a variate X to be normally distributed in two populations with common variance V and mean values m_1 and m_2, respectively. The ratio of the density functions is

$$\exp -\frac{1}{2V}[(X - m_1)^2 - (X - m_2)^2]$$

so that the logarithm of the ratio is

$$\frac{(m_1 - m_2)\,X}{V} - \frac{(m_1^2 - m_2^2)}{2V}$$

TABLE 6. ULNAR LOOP RIDGE COUNTS WHEN HOMOLOGOUS FINGER OF TWIN IS AN OUTER WHORL: RIGHT THUMB

Ridge count	Group 1		Group 3	
	Number	Cumulative per cent	Number	Cumulative per cent
1–	—	—	1	0.6
3–	2	2.2	1	1.3
5–	1	3.2	7	5.7
7–	—	3.2	6	9.4
9–	2	5.4	4	11.9
11–	3	8.6	7	16.4
13–	6	15.1	13	24.5
15–	6	21.5	23	39.0
17–	21	44.1	26	55.3
19–	20	65.6	28	73.0
21–	10	76.3	19	84.9
23–	11	88.2	15	94.3
25–	8	96.8	6	98.1
27–	2	98.9	2	99.4
29 and over	1	100.0	1	100.0
TOTAL	93	100.0	159	100.0
Median	20		18	

TABLE 7. DIFFERENCES OF ULNAR LOOP RIDGE COUNTS ON HOMOLOGOUS FINGERS OF TWINS: RIGHT THUMB

Difference in ridge count	Group 1		Group 3	
	Number	Cumulative per cent	Number	Cumulative per cent
0	39	12.5	20	8.2
1	63	32.6	35	22.5
2	55	50.2	30	34.7
3	49	65.8	35	49.0
4	35	77.0	28	60.4
5	22	84.0	15	66.5
6	18	89.8	17	73.5
7	8	92.3	15	79.6
8	7	94.6	12	84.5
9	7	96.8	9	88.2
10	4	98.1	5	90.2
11	3	99.0	10	94.3
12	2	99.7	3	95.5
13	1	100.0	4	97.2
14	—	100.0	2	98.0
15	—	100.0	1	98.4
16	—	100.0	1	98.8
17	—	100.0	1	99.2
18	—	100.0	1	99.6
19	—	100.0	—	99.6
20	—	100.0	1	100.0
TOTAL KNOWN	313	100.0	245	100.0

TABLE 8. COEFFICIENTS OF LINEAR EQUATIONS FOR RIDGE-COUNT SCORES: LOOP WITH OTHER PATTERN ON HOMOLOGOUS DIGIT OF TWIN

	Pattern of homologous digit	Constant term	Coefficient of loop ridge count
Ulnar loop on digit			
Thumb (either)	Arch	248	−3.34
Index (either)	Arch	228	−2.79
Middle (either)	Arch	225	−1.83
Ring (either)	Arch	241	−2.51
Index (either)	Tented arch	221	−2.48
Middle (either)	Tented arch	216	−1.76
Ring (either)	Tented arch	234	−3.58
Right thumb Left thumb	Outer whorl Inner whorl	174	1.75
Right index Left index	Outer whorl Inner whorl	153	3.76
Right middle Left middle	Outer whorl Inner whorl	149	4.07
Right ring Left ring	Outer whorl Inner whorl	178	1.56
Right little Left little	Outer whorl Inner whorl	183	1.40
Right index Left index	Inner whorl Outer whorl	184	1.97
Radial loop on digit			
Index (either)	Tented arch	214	−1.32

TABLE 9. COEFFICIENTS OF LINEAR EQUATIONS FOR RIDGE-COUNT SCORES: LOOPS OF SAME KIND ON HOMOLOGOUS DIGITS OF BOTH MEMBERS

	Constant term	Coefficient of difference of ridge counts
Ulnar loops on digits		
Thumb	220	−5.16
Index	214	−4.01
Middle	218	−4.76
Ring	219	−4.63
Little	219	−5.54
Radial loops on digits		
Index	213	−2.96

to the 2,805 pairs for which measurements were made at an interval of less than a year, a stepwise regression (Efroymson, 1960) was performed for the total fingerprint score on the four variables: height difference, reciprocal of height difference, weight difference as an absolute number, and weight difference with sign as positive if in the same direction as the height difference, and otherwise negative. The first two variables to be entered were the height

TABLE 10. RELATION OF HEIGHT DIFFERENCE AND WEIGHT DIFFERENCE TO SEROLOGICAL DIAGNOSIS OF ZYGOSITY

Restricted to Measurements Made at an Interval of Not More than One Year

	Laboratory diagnosis									
	Total		Concordant serologically, probably MZ		Discordant serologically, DZ		Concordant serologically, probably DZ		Not classified	
	Number	%	Number	%	Number	%	Number	%	Number	%
Height difference (inches)										
Same	90	32.6	55	45.5	25	20.3	3	25.0	7	35.0
1	109	39.5	53	43.8	39	31.7	6	50.0	11	55.0
2	42	15.2	12	9.9	29	23.6	—	—	1	5.0
3	14	5.1	—	—	11	8.9	2	16.7	1	5.0
4 or more	21	7.6	1	0.8	19	15.4	1	8.3	—	—
TOTAL	276	100.0	121	100.0	123	99.9	12	100.0	20	100.0
Weight difference (pounds)										
0–4	88	31.9	50	41.3	32	26.0	1	8.3	5	25.0
5–9	68	24.6	36	29.8	21	17.1	3	25.0	8	40.0
10–14	47	17.0	19	15.7	20	16.3	4	33.3	4	20.0
15–19	36	13.0	11	9.1	20	16.3	3	25.0	2	10.0
20 or more	37	13.4	5	4.1	30	24.4	1	8.3	1	5.0
TOTAL	276	99.9	121	100.0	123	100.1	12	99.9	20	100.0

difference and absolute weight difference, the other two having no significant independent information. Table 10 shows the relations between zygosity and height difference and absolute weight difference individually. Similarly, a new eye- and hair-color score was obtained from the regression of the *F* score on the eye-color and hair-color combination classes for the whole sample of 3,898 pairs, and finally a total "anthropometric score" (*AS*) was defined, simply, as the sum of the eye- and hair-color and height-weight scores. The *AS* variable was then used to find a better way to combine the individual digit scores than merely averaging all ten of them. Five scores, *D*1 to *D*5, were obtained by summing the scores for the two thumbs, index fingers, etc. A regression was then taken of the *AS* on the five digit scores, and numbers proportional to the regression coefficients were used to obtain, finally, the S1 score, based on fingerprints alone:

$$S1 = .25 \times D1 + .23 \times D2 + .19 \times D3 + .16 \times D4 + .17 \times D5 - 400$$

The value 400 was subtracted to keep the S1 scores in the range 350 to 450.

The following additional scores were then calculated:

S3, which summarizes fingerprint plus all anthropometric information for those pairs for whom the latter is known;

S4, which summarizes fingerprint plus eye- and hair-color information for those pairs for whom the latter is known; and

S5, which summarizes all of the information that may be available for a pair.

The performance of the scoring system was subject to check by comparison with the diagnoses made on the panel previously described. It turned out that, for three of the 257 veteran pairs, the fingerprints were not of satisfactory technical quality so that the diagnoses could be compared on 335 pairs only (254 pairs from this study and 81 pairs from the study of Sutton *et al.*, 1962). Results are shown in Tables 11 and 12. The performance of a summary classification based on S5 is shown in Table 13. Forty-eight pairs, or 14.3% of the 335 pairs, fell into a middle, unclassifiable group, while 132 pairs were classed as monozygotic and 155 as dizygotic. Within each of these latter two groups, 12–13% of the diagnoses were contradicted by the serological diagnoses, 81–83% were confirmed, and the remainder were not classified. Ignoring those pairs not classified serologically, the diagnoses based on the S5 score were 87% correct, using the serological diagnosis as a standard.

THE RESPONSE OF THE SUBPANEL OF ESTABLISHED ZYGOSITY TO A QUESTIONNAIRE REGARDING ZYGOSITY

Over their lifetimes, like-sexed male twins not only have abundant opportunity to decide whether they are more alike than most brothers but also are exposed to numerous lay and even professional opinions concerning zygosity. However, there appear to have been only three studies in which the opinions of adult twins concerning zygosity were systematically compared with the results of blood grouping studies (Cederlöf *et al.*, 1961; Harvald and Hauge, 1965; Nichols and Bilbro, 1966). The present investigation provided a third opportunity to test both the accuracy of diagnosis and the response rate to be expected from adult male twins such as comprised the subsample selected for detailed studies of zygosity. When the twins were interviewed no opinion was expressed concerning zygosity by the field worker. When the field work was finished, the following letter was sent to each member of the 232 pairs upon whom studies were complete:

> Last summer you were contacted by a member of this Department in connection with our twin study. At that time we promised to send you a complete report of the results of our blood typing, with our opinion as to whether you were an identical or fraternal twin. The blood studies have taken longer than anticipated, but we are finally ready to send our reports out.
>
> I would like at this point to request a small favor of you. It has been said that twins themselves know whether they are identical or not, and that their statements are more accurate than scientific tests. Before we send you *our* opinion, would you be so kind as to check your opinion on the enclosed post card and drop it in the mail box. Identical twins, as you know, are almost completely alike, whereas the other (fraternal) type are no more alike than any pair of brothers.

It is apparent that the letter contained a minimum of explanatory material; this was deliberate, to set a baseline. It seems possible a somewhat more detailed letter with specific questions might have assisted in the discrimination. The response rate was 89.9%. This is a rather good rate, due perhaps to the previous contact, the challenge implied in the letter, and the fact that the individuals concerned were to receive our opinion and the results of the

TABLE 11. RELATION OF FINGERPRINT SCORE (S1) TO SEROLOGICAL DIAGNOSIS OF ZYGOSITY

	Laboratory diagnosis									
	Total		Concordant serologically, probably MZ		Discordant serologically, DZ		Concordant serologically, probably DZ		Not classified	
*S*1 Score	Number	%	Number	%	Number	%	Number	%	Number	%
Less than 350	3		1		2		—		—	
350–359	5				4		1		—	
360–369	7	17.6		1.4	7	35.1	—	28.6	—	—
370–379	21				20		1		—	
380–389	23		1		20		2		—	
390–399	44	13.1	11	7.6	25	16.6	5	35.7	3	12.0
400–409	59	17.6	20	13.8	31	20.5	2	14.3	6	24.0
410–419	76	22.7	38	26.2	25	16.6	1	7.1	12	48.0
420–429	60	17.9	41	28.3	14	9.3	1	7.1	4	16.0
430–439	31		27		3		1		—	
		11.0		22.8		2.0		7.1		—
440 and over	6		6		—		—		—	
TOTAL	335	99.9	145	100.1	151	100.1	14	99.9	25	100.0

TABLE 12. RELATION OF SUMMARY FINGERPRINT AND ANTHROPOMETRIC SCORE (S5) TO SEROLOGICAL DIAGNOSIS OF ZYGOSITY

	Laboratory diagnosis									
	Total		Concordant serologically, probably MZ		Discordant serologically, DZ		Concordant serologically, probably DZ		Not classified	
S5 Score	Number	%	Number	%	Number	%	Number	%	Number	%
Less than 320	1		1		—		—		—	
320–369	31	20.6	—	1.4	29	41.7	2	28.6	—	—
370–379	37		1		34		2		—	
380–389	23	6.9	3	2.1	16	10.6	4	28.6	—	—
390–399	31	9.3	7	4.8	17	11.3	3	21.4	4	16.0
400–409	32	9.6	8	5.5	22	14.6	—		2	8.0
410–419	48	14.3	18	12.4	19	12.6	1	7.1	10	40.0
420–429	43	12.8	27	18.6	10	6.6	1	7.1	5	20.0
430–439	44		38		1		1		4	
440–449	33	26.6	30	55.2	3	2.6	—	7.1	—	16.0
450 and over	12		12		—		—		—	
TOTAL	335		145		151		14		25	

TABLE 13. COMPARISON OF DIAGNOSES OF ZYGOSITY BASED ON FINGERPRINT AND ANTHROPOMETRIC SCORE (S5) WITH THOSE BASED ON SEROLOGY

	Laboratory diagnosis							
	Total		Monozygotic		Dizygotic*		Not classified	
S5 diagnosis	Number	%	Number	%	Number	%	Number	%
Monozygotic (420 and over)	132	100.0	107	81.1	16	12.1	9	6.8
Dizygotic (less than 410)	155	100.0	20	12.9	129	83.2	6	3.9
Not classified (410–419)	48	100.0	18	37.5	20	41.7	10	20.8
TOTAL	335	100.0	145	43.3	165	49.2	25	7.5

*Includes those concordant serologically but with significant morphological differences.

TABLE 14. THE RESPONSE OF 232 PAIRS OF TWINS TO A QUESTIONNAIRE REGARDING ZYGOSITY

(?) Indicates No Response.

	Laboratory diagnosis		
Opinion of twin pair	Concordant serologically, probably MZ	Concordant serologically, probably DZ	Discordant serologically, DZ
MZ-MZ	71	1	1
MZ-DZ	9		5
DZ-DZ	4	4	95
MZ-(?)	12		
DZ-(?)	3	2	20
(?)-(?)	4		1
TOTAL	103	7	122

blood typing studies *after* the response. It seems unlikely that a similarly high response would be forthcoming in a study where there were no previous contacts.

The results of the questionnaire are given in Table 14. Of the total of 417 responses, there were 388 (93.0%) which agreed with the laboratory diagnosis. Of 195 pairs where both members responded, the twins agreed and in our opinion were correct in 170 instances (87.2%). Expressed in terms of individual twins, the error rate where both responded was 6.4%. In 37 instances where only one twin responded, the opinion coincided with that of the laboratory in 34 cases, an error rate of 8.1%. Thus, the error rate was of the same magnitude when only one twin responded as when both responded. More monozygotic twins erred in the belief they were dizygotic (10.9%) than dizygotic twins erred in the belief they were monozygotic (3.2%). This may reflect a mistaken belief that identical twins should demonstrate complete concordance in such traits as height and weight. However, we must recognize the possibility that some twin pairs who are concordant serologically and morphologically and classed by us as monozygotic are nevertheless dizygotic and that the opinion of the twin pairs is as valid

as our own. Because, however, from the statistical standpoint there is already something of an excess of serologically concordant twins who were felt to be dizygotic, acceptance of the foregoing thought would result only in a further departure from expectation.

Cederlöf *et al.* (1961), with a similar comparison of serological findings and the questionnaire approach in Sweden, did not ask the like-sexed twins what kind they considered themselves to be, but rather asked a series of questions on which they then based an opinion. Because on some occasions the answers of the twins not only disagreed (as in our study) but were internally conflicting, a number of different evaluations are possible. Serological studies included the A_1A_2BO, MN, Rhesus, haptoglobin, and Gm systems, with the median probability that monozygous twins would be concordant estimated at about 96%. On the basis of what they regard as the best method for handling their questionnaire results, for 92% of the twins the replies of *both* twins were consistent with the serological results, in good agreement with our own findings. Harvald and Hauge (1965) investigated the agreement between blood groupings and questionnaire findings in a Danish sample of 165 like-sexed pairs, using a questionnaire similar to that of Cederlöf *et al.* (1961). It was calculated that serological concordance carried a probability of monozygosity of 98%. They write:

> Out of 78 pairs which had been classified as MZ according to the questionnaire, all except one pair presented no difference with regard to blood or serum group. Eighty pairs considered DZ according to the questionnaire showed one or more intrapair blood group differences in 76 cases, whereas the remaining four pairs had identical blood groups. Out of the total 165 pairs, seven had been classified as being of uncertain zygosity on the basis of the questionnaire. Four of these had identical blood and serum groups. Thus, it turns out that our diagnostic procedure is sufficiently reliable.

Again there would appear to be essential agreement with the results of our own approach.

THE ACCURACY OBTAINABLE BY A COMBINATION OF QUESTIONNAIRE AND DISCRIMINANT FUNCTION

It has been seen that the twins' own opinion is a fairly good guide to the diagnosis of zygosity, better than the laboriously derived dermatoglyphic score. It seems natural, then, to attempt to combine the two kinds of information. Table 15 shows the relations between the three factors of (1) the twins' opinions, (2) the S5 score (based on fingerprints and, where available, recorded anthropometrics), and (3) the diagnosis based on serological examination. Because, for those pairs in which only a single reply was obtained, agreement with the serological diagnosis was about as good as for those in which both replied identically, the pairs have been sorted into just three groups, on the basis of their opinions: Monozygotic, when either both so replied or when this was the only answer received; Dizygotic, similarly defined; and Questionable, when both answered but disagreed or when neither answered.

It is immediately apparent from Table 15 that the three systems of classi-

TABLE 15. RELATION BETWEEN TWINS' OPINION OF ZYGOSITY, S5 ZYGOSITY SCORE, AND LABORATORY DIAGNOSIS

Twins' opinion and S5 Zygosity Score	Total	Laboratory diagnosis			
		Concordant serologically, probably MZ	Concordant serologically, probably DZ	Discordant serologically, DZ	Per cent MZ
Total	229	101	7	121	
Monozygotic [MZ-MZ or MZ-(?)]	84	82	1	1	97.6
430 or more	43	42	1	—	97.7
420–429	16	15	—	1	93.8
410–419	9	9	—	—	100.0
390–409	11	11	—	—	100.0
Less than 390	5	5	—	—	100.0
Questionable [MZ-DZ or (?)-(?)]	18	12	—	6	66.7
430 or more	7	6	—	1	85.7
420–429	3	3	—	—	100.0
410–419	3	1	—	2	33.3
390–409	4	2	—	2	50.0
Less than 390	1	—	—	1	0.0
Dizygotic [DZ-DZ or DZ-(?)]	127	7	6	114	5.5
430 or more	5	4	—	1	80.0
420–429	9	1	1	7	11.1
410–419	14	2	—	12	14.3
390–409	28	—	—	28	0.0
Less than 390	71	—	5	66	0.0

fication, although not identical, are very highly correlated. Further, it seems clear that if the twins state that they are MZ, their opinion is decisive; in only two of 84 such pairs was the laboratory diagnosis DZ, and both had S5 scores indicative of MZ status. At the other extreme, if the twins' statement was DZ, the laboratory diagnosis was usually DZ also, only 7/127 pairs, or 5.5% being contradicted. However, of five such pairs with an S5 score of 430 or more, four were judged to be MZ on the basis of concordant serology and no obvious anthropometric differences. Moreover, the S5 score was able to discriminate usefully among the 18 pairs for which twins' diagnoses were not available. Table 16 shows the final diagnostic grouping based on the combination of S5 and the twins' opinions. Only seven, or 3.1% of the 229 pairs, were unclassifiable. Using the laboratory diagnosis as a standard, the errors were only 4.0% for those classified as MZ and 2.4% for those classified as DZ.

The error rates, 4.0% and 2.4%, are relatively small, yet it is necessary to consider explicitly what influence such errors may have on the estimate of heritability for any trait (or disease) to be studied. A main purpose of the panel being developed is the study of chronic disease, and consideration of

TABLE 16. RELATION BETWEEN DIAGNOSIS BASED ON COMBINATION OF TWINS' OPINIONS AND S5 ZYGOSITY SCORE AND LABORATORY DIAGNOSIS

Combined diagnosis		Total	Laboratory diagnosis: Concordant serologically, probably MZ	Laboratory diagnosis: Concordant serologically, probably DZ	Laboratory diagnosis: Discordant serologically, DZ	Per cent MZ
Total		229	101	7	121	44.1
Monozygotic		99	95	1	3	96.0
Opinion	*S5 Score*					
MZ	Any	84	82	1	1	97.6
Questionable	420 or more	10	9	—	1	90.0
DZ	430 or more	5	4	—	1	80.0
Questionable		7	3	—	4	42.9
Opinion	*S5 Score*					
Questionable	390–419	7	3	—	4	42.9
Dizygotic		123	3	6	114	2.4
Opinion	*S5 Score*					
Questionable	Less than 390	1	—	—	1	0.0
DZ	Less than 430	122	3	6	113	2.5

the heritability of such a disease as gastric carcinoma involves a number of complex questions regarding age of occurrence, incompleteness of observation on men who *at last report* were free of disease, etc. For these reasons, among others, as pointed out by the report of the World Health Organization (1966), it is not clear that the simple concept of "concordance" is appropriate to these problems, let alone the heritability index. However, we shall limit ourselves here to consideration of the effect of errors in zygosity diagnosis on the commonly used measure of heritability, which may be expressed as

$$H = \frac{P(C|MZ) - P(C|DZ)}{1 - P(C|DZ)} \tag{1}$$

where $P(C|MZ)$ is the relative frequency of concordance in MZ pairs, and $P(C|DZ)$ in DZ pairs. Primes on these variables denote that they are calculated using zygosity diagnoses that are subject to error. Then

$$H' = \frac{P'(C|MZ) - P'(C|DZ)}{1 - P'(C|DZ)} \tag{2}$$

and, in general, will not be equal to H. Let $E(MZ)$, $E(DZ)$ be the proportions of pairs that are classified erroneously as MZ and DZ, respectively, .040 and .024 for the combination of S5 and the twins' statement.

From the definitions of $E(MZ)$, $E(DZ)$ it follows that, on the average, assuming that errors in diagnosis of zygosity are independent of concordance or discordance for the trait,

$$P'(C|MZ) = [1 - E(MZ)] \times P(C|MZ) + E(MZ) \times P(C|DZ)$$

and $$P'(C|DZ) = E(DZ) \times P(C|MZ) + [1 - E(DZ)] \times P(C|DZ)$$

so that

$$P'(C|MZ) - P'(C|DZ) = [1 - E(MZ) - E(DZ)] \times [P(C|MZ) - P(C|DZ)]$$

TABLE 17. EFFECT OF ERRORS IN ZYGOSITY DIAGNOSIS ON ESTIMATE OF HERITABILITY

H using perfect diagnoses	H' using diagnoses subject to error*	H'/H
1.000	.959	.959
.800	.763	.954
.600	.570	.950
.400	.378	.945
.200	.188	.941
.100	.094	.938

*Errors are assumed to be 4.0% of pairs classed as MZ and 2.4% of pairs classed as DZ.

$$\begin{aligned} \text{and } 1 - P'(C|DZ) &= [1 - P(C|DZ)] - E(DZ) \times [P(C|MZ) - P(C|DZ)] \\ &= [1 - P(C|DZ)] - E(DZ) \times H \times [1 - P(C|DZ)] \\ &= [1 - P(C|DZ)][1 - E(DZ) \times H] \end{aligned}$$

Finally,

$$H' = H \times \frac{1 - E(MZ) - E(DZ)}{1 - E(DZ) \times H} = \frac{(.936) \times H}{1 - (.024) \times H}$$

$$\text{and } H = \frac{H'}{1 - E(MZ) - (1 - H')E(DZ)} = \frac{H'}{.936 + (.024) \times H'}$$

From Table 17 it can be seen that with diagnostic error rates as small as those demonstrated here, H' is about 4–6% too low as compared with H. However, H can be estimated from the value of H', though naturally the statistical error in H as obtained via H' will be larger than if H is directly estimated using an infallible system of zygosity determination. The relative efficiency of the estimate of H obtained in this way is a complicated function of the two error rates and the two probabilities of concordance in twins of both kinds. However, for the error rates that concern us (4.2% and 2%) the relative efficiency was calculated by computer for different values of H ranging from zero to 0.50, using various combinations of values for $P(C|MZ)$ and $P(C|DZ)$ less than 0.500, and for the values tried efficiency was in the range 0.88 to 0.92. That is, the errors in diagnosis are equivalent in cost, statistically, to about 10% of the sample size. It can be expected, therefore, that the loss of accuracy introduced by diagnostic errors of about 4.0% and 2.4% will not seriously interfere with the objectives for the panel.

ONGOING INFORMATION RETRIEVAL

Information being placed in the Registry can, for convenience, be divided into three types: (1) identifying information, required to decide whether a pair should be included in the Registry and to locate a man or his records subsequently, (2) information required for zygosity diagnosis, and (3) follow-up information concerning medical history since entry into military service.

Identifying Information

The original documents for every pair are the *birth certificates,* which show date and place of birth and parents' names.

Every name was searched through the VA Master Index during the period 1958–1959. At this time there was obtained the military service number, the VA claims number (issued to the 75% of World War II veterans who had disability claims, VA hospitalizations, or other benefits), and the VA insurance number for GI insurance (issued to about 98% of World War II veterans).

The reasons for obtaining these identifying numbers varied. The military service number is required for entry to the files of military personnel and medical records at the St. Louis records center. The insurance number provides an entry to the VA Insurance files, which are often valuable as a source of current addresses. Finally, the claim number is required to obtain access to the VA claims folder which contains notices of VA hospitalizations within the VA hospital system; admissions to other hospitals as a beneficiary of the VA; and the clinical records for admission to military hospitals while in service, if these records were requested from the military by the VA in connection with the adjudication of a claim for a veteran's benefit based on an injury or disease occurring while in service. The claims folder also shows the address of the veteran at the time of his last contact with the VA.

Information Required for Zygosity Diagnosis

The three kinds of information used in zygosity diagnosis are obtained from three different sources. The *fingerprints* of the twins are obtainable only from the FBI. Because of the press of regular work, at this writing, the FBI has been able to process only about one-third of the Register through its files. The *anthropometric data* (height, weight, eye color, and hair color recorded at the time of entry into service) are obtained from the individual military records. These have all been collected. The *twins' opinions* of zygosity are now being obtained by mail questionnaire, along with follow-up medical information to be described below. More than 15,000 completed questionnaires are in hand at this time (September, 1966).

Follow-Up Information

The *military records* show defects present at entry into service, the diagnoses for all hospitalizations or outpatient treatments in military medical facilities, the anthropometric data mentioned above, blood pressure as measured at the induction physical examination, marital status, education, and occupation. This information has been abstracted for all men in the Registry.

For the 24,767 men who had *VA claims folders,* VA clerks abstracted information on hospitalizations as a VA beneficiary, whether in the VA hospital system or otherwise, and also information about military hospitalizations for which the records had been transferred to VA. If the veteran was known to have died, the date, place, and cause of death were shown. If the veteran

had a disability rating, the rating was given, together with the conditions for which compensation was being paid.

The *questionnaires,* in addition to eliciting the twins' own opinions of zygosity, ask for a history of all episodes of hospitalization since separation from military service, including the name of the illness, year of onset, and name and location of the hospital. Each man is also asked for the address of his twin.

Finally, the fact of death is obtainable from the VAMI with 98% accuracy (Beebe, 1966), and mortality experience through 1964 has been obtained.

DISCUSSION

Despite the use of the so-called "twin method" by many investigators, beginning with Galton about 90 years ago, critics have repeatedly called attention to certain shortcomings of the method. The most serious problem, as we view it, is the implicit or explicit assumption that like-sexed fraternal twins experience a common environment to the same degree as do identical twins. This problem was considered by the recent WHO Meeting of Investigators on Methodology of Twin Studies, who concluded in their report (World Health Organization, 1966) that:

> . . . normal MZ twins can be presumed, in general, to share more features of environment and experience than do DZ twins. As long as the etiology of the disease is not fully defined, the possibility cannot be excluded that some item of experience shared by MZ partners is a determining factor. As a theoretical argument, this is almost impossible to refute.

However, the authors of the report go on to state that:

> In reality, most shared postnatal experiences of MZ twins are probably not qualitatively different from those shared by DZ partners or even sibs. Consequently a critical environmental factor that could explain a relatively high concordance in the MZ twins would probably raise the concordance in DZ twins well above that of other sib pairs . . .

and conclude that

> . . . with respect to genetic determinations, a comparison of concordance in MZ and DZ twins can do no more than to draw attention to the presumptive importance of genetic factors.

It must be said, however, that epidemiological studies of human populations, especially studies of chronic disease, whatever be their methodology, are subject to equivalent uncertainties. The difficulty is that such studies are almost necessarily observational rather than manipulative. The observer is unable to bring to bear those techniques of randomization and experimental control that can ensure certainty of conclusions. We believe therefore that, although the problem mentioned dictates cautious interpretation of results, twin studies nevertheless do have an important role to play.

Although epidemiologic studies using twins would appear to focus primarily on the genetic component of causation, attention should be drawn to the great potential usefulness of monozygotic pairs in the elucidation of specific environmental factors suspected as being of etiologic importance to particular

diseases. An example would be MZ pairs discordant for smoking in relation to respiratory cancer.

If it is accepted that twin studies are an essential component of efforts to unravel the genetic and environmental threads in epidemiology, what are the advantages and disadvantages of the roster of veteran twins in relation to other twin panels? Two restrictions come immediately to mind: No females are represented, and much disease that occurs early in life has been eliminated by the condition that both members of each pair not only must have survived but must have passed the physical examination for entry into military service. Fortunately, these restrictions, although they do narrow the scope of applicability for the panel, do not affect its suitability for the purposes for which it is primarily intended, namely, elucidation of the role of genetic factors in the etiology of chronic disease. On the other hand, the panel possesses very important advantages: It is very large, and can be expected to include a reasonable number of men diagnosed as having even relatively rare conditions. It is essentially complete for the states and years of birth used; continuing mortality ascertainment is essentially complete and relatively inexpensive; much information about morbidity is routinely obtainable from hospitalizations within military or VA hospitals; and the problem of keeping track of current addresses, although not solved, is made much easier by the use of applicable centralized government files. This last point is of considerable importance: The population of the United States, particularly young adult males, is so highly mobile that it is almost impossible to maintain the integrity of a large list of persons without such aids.

It is noteworthy that the method of study applicable to the panel is that of prospective ascertainment, in distinction to collections of twin pairs that have come to notice only because at least one of the pair has been recognized as having some particular disease. It is obvious that, because of the relation between detection of disease or concordance and selection for study, such series have but limited usefulness for inference about populations, except perhaps for conditions for which all identical pairs are concordant. Such problems of bias are avoided in this panel.

FUTURE WORK

Now that the task of constructing the panel, including the definition of methods of zygosity diagnosis, has been or soon will be essentially completed, how will the panel actually be used? The panel is designed to be a resource, available under suitable safeguards to qualified investigators who have significant problems for which this material would be of value.

(1) It is planned to tabulate periodically the numbers of men and of concordant and discordant pairs of each kind for every disease as ascertained from records and questionnaires and to publish summaries of these data.

(2) It is expected that, on the basis of these summaries, special studies will be undertaken by investigators with special interests. An example might

be the detailed examination and evaluation of the co-twins of those twins who have suffered coronary attacks or had strokes.

Access to the panel will be monitored by the Committee on Epidemiology and Veterans Follow-up Studies, Division of Medical Sciences, National Academy of Sciences–National Research Council. The Committee's review will ensure that accepted proposals are scientifically valid, that there is no unnecessary duplication of effort, and that the future co-operation of the twins is not endangered either by oversolicitation or by unnecessarily prolonged or unpleasant methods of examination. The Committee expects that the costs of maintenance of the panel, and perhaps of providing records service to investigators, will be defrayed by funds that will be available to the Committee. But it will be the responsibility of each outside investigator himself to arrange for any funding necessary for his own part in any study.

It is expected that an initial index will be ready early in 1967 and that outside requests for access to the panel can be considered at that time.

SUMMARY

A panel of 16,000 pairs of twins, all of whom are veterans, has been assembled by means of matching VA files against the names given on records of multiple births in 42 vital statistics offices for the years 1917–1927. Records of physical examination and defects noted at entry into service have been abstracted from military files. Military and VA records of hospitalizations have been searched and diagnoses obtained. Copies of fingerprint cards have been obtained from the FBI for about half the panel at this time.

Considerable effort has gone into devising methods of zygosity diagnosis suitable for such a large file. The methods explored have included analysis of fingerprints, recorded information about height, weight, eye color, and hair color, and the twins' own opinions, as obtained by mail questionnaire. Proposed methods of diagnosis were validated by comparison with diagnoses made on a subset of 257 pairs from whom blood samples were obtained and typed with respect to nine genetic systems. An additional group of 81 nonveteran twin pairs was also used in the validation. The twins' opinions were the single most reliable indicator of zygosity, having an average error of about 4.3%. Diagnoses based on fingerprints alone had an average error of 22.6%, but when the fingerprint information was supplemented by information on height and weight differences and eye and hair color, as shown in the military records, the average error dropped to about 13%. The best diagnoses were based on both the recorded information and the twins' opinions; for this combination, the error was only 3.2%. It appears that about 44% of the pairs in the panel are MZ and 56% DZ.

The panel is being readied as a resource that may be used, under certain conditions, by interested investigators. The panel is expected to have the greatest utility for studies directed at elucidation of genetic factors in chronic disease. Use of the panel will be monitored by the Committee on Epidemiology and Veterans Follow-up Studies of the Division of Medical Sciences, National Academy of Sciences–National Research Council.

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APPENDIX 1

The number of pairs observed in a given category may be considered to be distributed approximately as a Poisson variable. The problem is, then, to find, if possible, an unbiased estimator of log m, where m is the expectation of a Poisson variable, given a single observation x.

The maximum likelihood estimator of log m is log x, but because there is a positive probability that x is zero, this estimator has infinite expected value. It seems natural, therefore, to try an estimator of the form

$$u = \log (x + c)$$

Then

$$u - \log m = \log [(x + c)/m] = \log [1 + (x - m + c)/m]$$

If m is reasonably large, the absolute value of $x - m$ will not often exceed $3m^{1/2}$, so that $(x - m)/m$ will, with high probability, not exceed $3/m^{1/2}$, or will be less than one for m more than nine. In any event, this suggests expanding the logarithm in a power series and taking the expected value term by term. If the resulting series is arranged in descending powers of m, there is obtained:

$$E(u) - \log m = \frac{1}{m}(c - \tfrac{1}{2}) - \frac{1}{6m^2}(3c^2 - 6c - 2) + \ldots$$

This suggests that at least for large values of m, the use of $c = \frac{1}{2}$ will be satisfactory. The actual performance of this estimator is shown below for certain values of m:

m	$E \log (x + \frac{1}{2})$	$\log m$	Bias
0.001	−0.692	−6.908	6.216
0.01	−0.682	−4.605	3.923
0.1	−0.586	−2.303	1.717
1.0	0.169	0.0	0.169
2.0	0.719	0.693	0.026
3.0	1.101	1.099	0.002
5.0	1.607	1.609	−0.002

APPENDIX 2

The problem is to fit

$$S_i = a + bX_i$$

given observed pairs S'_i, X_i where each S'_i is of the form

$$K + \log (m'_i + \tfrac{1}{2}) - \log (d'_i + \tfrac{1}{2})$$

The m'_i, d'_i may be assumed to be Poisson variates with expected values m_i, d_i, respectively. For values of m_i that are not too small to permit expansion of the logarithms in a series of inverse powers of m'_i, calculation shows that

$$E\,[\log (m'_i + \tfrac{1}{2}) - \log m_i]^2 = 1/m_i + \tfrac{1}{2}m_i^2 + \ldots$$

Hence the mean square error in $\log (m'_i + \tfrac{1}{2}) - \log (d'_i + \tfrac{1}{2})$ as an estimate of $\log m_i - \log d_i$ is, for large m_i and d_i, approximately $1/m_i + 1/d_i$, and the reciprocal, $m_i d_i/(m_i + d_i)$ is therefore a reasonable weight for the ith point in the curve fitting. This value was estimated by the value

$$W_i = \frac{(m'_i + \tfrac{1}{2})(d'_i + \tfrac{1}{2})}{(m'_i + d'_i + 1)}$$

for which the expected value, up to terms of degree zero in m_i and d_i is

$$E(W_i) = \frac{m_i d_i}{m_i + d_i} + \left(\tfrac{1}{2} - \frac{2\,m_i d_i}{(m_i + d_i)^2}\right)$$

The second term in the above expression vanishes if $m_i = d_i$.

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