The Hereditary Abilities Study: Genetic Variation in Human Biochemical Traits

H. ELDON SUTTON,[1] ELVIRA GIL DE LAMADRID AND MARIE B. ESTERER

Institute of Human Biology and Department of Human Genetics, The University of Michigan, Ann Arbor

DURING THE PAST DECADE, a considerable interest has centered around the existence of biochemical variability in man. In addition to the well-known examples of inherited differences in red cell antigens, there are now known to exist inherited variations in a number of proteins, such as hemoglobin, haptoglobin, transferrin, the Gm factor, and albumin. In the examples listed, it has been possible more or less directly to associate the phenotype observed with variation at one or relatively few loci. Presumably this is related to the fact that the variable products are rather closely related to the primary gene products.

The ultimate function of most proteins, however, is to maintain small molecular weight substances in the proper metabolic balance. These small metabolites should therefore reflect variation in proteins, particularly enzymes, but one would expect them also to be influenced by environment. Here again, one can cite examples in which single genes exert marked influence on one or a few metabolities. Phenylketonuria and galactosemia are instances which are so well known as not to justify description. In both these cases, even though the genetic factors may be the principal agents of variation, the influence of environment is also well established.

Both phenylketonuria and galactosemia represent conditions in which enzyme activity is essentially completely absent. However, we know from studies of microorganisms and indeed from studies of human hemoglobin variants that mutant proteins frequently to retain some of their enzyme activity. In such cases, the mutant individual may well possess a phenotype within the normal range of variation. Recent studies of a number of loci suggest that a large number of alleles may be present at any one locus, most of them functionally adequate although not necessarily functionally equal. It is presumably these inequalities which make up the inherited variation so apparent among the normal population.

A number of studies have been carried out which demonstrate the very large range of variability to be found in the levels of metabolites in various biological fluids. These studies have particularly been aided by the development of paper chromatographic procedures, which can be readily applied in the

[1]Present address: Department of Zoology, The University of Texas, Austin.

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detection of large numbers of substances in fairly simple operations. Few of these studies have been designed, however, to separate genetic from environmental factors. This distinction is not readily made in most cases, since the effects of the environment are neither equal nor constant. The twin study method does offer an effective approach to this problem. It has not been applied, however, to studies of metabolic variation except on a limited basis.

The principal study which has been reported was that carried out at Columbia University (Berry *et al.*, 1955; Gartler *et al.*, 1955) in which chromatographic procedures were applied to urine samples from 27 pairs of twins. Amino acids were the principal substances measured. In that study it was reported that significant hereditary variability is found among several of the amino acids.

The present report deals with biochemical and hematological studies of a series of twins who were investigated as part of the Hereditary Abilities Study of The University of Michigan. The chemical measures used were largely based upon paper chromatographic procedures. Biological fluids investigated include urine, blood, and saliva.

METHODS

The subjects consisted of 82 sets of like-sexed twins 12 to 20 years of age. There were 45 sets of monozygotic twins and 37 dizygotic. A more detailed description of the subjects and of the diagnosis of zygosity is given in a previous paper (Sutton *et al.*, 1962). Three urine samples were collected from each individual. The first, designated sample 1, was a timed morning sample collected in the laboratory over approximately a two hour period. The other two samples, labeled 2 and 3, were first morning samples collected subsequently with intervals of at least several days between samples. At the beginning of the time period for collection of sample 1, usually about 8:30 A.M., a venous blood specimen was also collected. The blood sample was divided into two lots, one being placed in dried citrate to facilitate hematological studies and the other allowed to clot. As soon as the clot had retracted, serum was removed and frozen for further analysis. Following collection of the blood sample, saliva was also collected without stimulation, usually 2 to 3 ml. The pH of the saliva was measured immediately with a glass electrode meter, following which the saliva was placed in boiling water for 10 minutes to inactivate enzymes. It was then frozen for subsequent testing of secretor status and for chromatographic studies.

Hematologic measures were made by standard clinical techniques. Eosinophils were counted directly in a Speirs-Levy eosinophil counting slide in order to increase the accuracy. The circulating levels of other leukocytes were obtained by multiplying the differential white count by the total white cell count.

Creatinine was measured by the alkaline picrate method. In the case of serum creatinine, deproteinization was carried out by the method of Brod and Sirota (1948). Optical density measurements of the urine were made in a Beckman Model B spectrophotometer at 420 mμ. Two milliliters of urine were adjusted to neutrality at room temperature and diluted to a final volume of 4 ml. The diluted samples were centrifuged to remove any suspended matter, the supernatant being used to read the optical density. Specific gravity of urine was measured with a conventional urine hydrometer.

Amino acid chromatograms were prepared by procedures which have become conventional. Whatman No. 1 filter paper was used in the ascending method, and only two-dimensional chromatograms were prepared. The size of the filter paper was 23 cm. by 28 cm. A phenol solvent was used for the first direction, and lutidine was used for the second, both prepared according to the direction of Berry *et al.* (1951).

Urine chromatograms were prepared by adding an aliquot of untreated urine contain-

ing 50 micrograms of creatinine to the filter paper. The sample was added 5 microliters at a time, care being taken that the spot was well dried between additions. In the case of serum it is necessary to remove most of the protein before preparing the chromatogram, otherwise the amino acid spots will not separate. This was achieved by adding three volumes of 95 per cent ethanol to one volume of blood serum, centrifuging down the precipitate, and using the supernatant. One hundred μl. of supernatant were used as the test amount, assumed to be equivalent to 25 μl. of serum. While it is recognized that small amounts of amino acids may be lost by absorption to the precipitate, such error should not influence the statistical analysis for which these results were intended. In the case of saliva, 100 μl. of the heated material was added to each chromatogram, also in 5 μl. aliquots. The brief heat treatment designed to inactivate enzymes is not usually adequate to produce much precipitation of proteins. However the protein concentration of saliva is sufficiently low so that there is no interference with amino acid chromatograms.

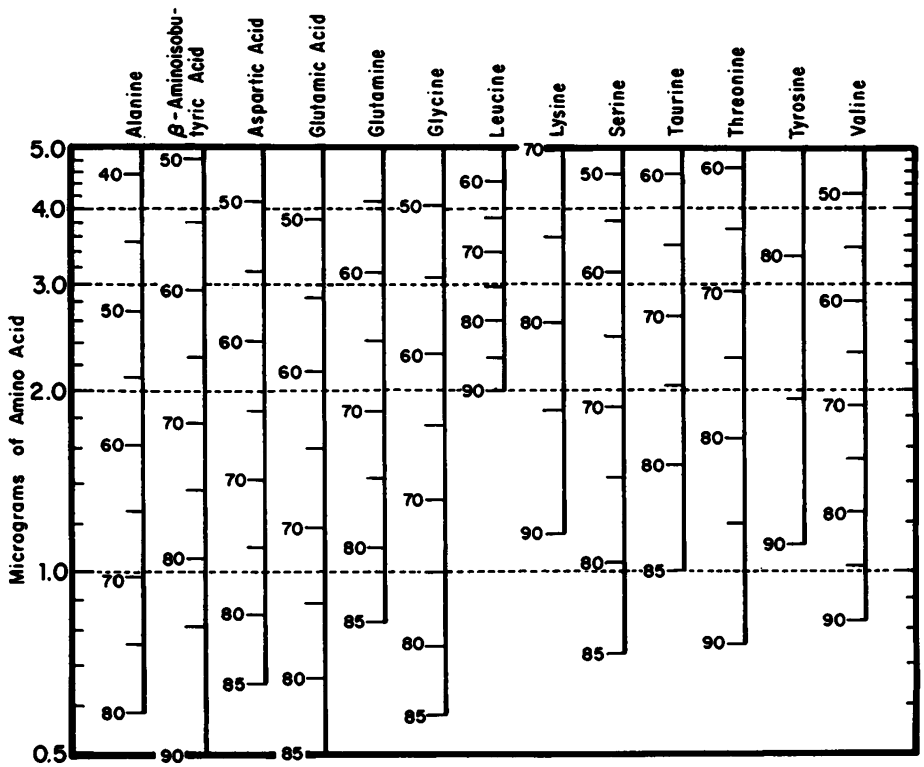

FIG 1. Scale showing relationship of reflectance of amino acid spots on two-dimensional chromatograms to amounts of amino acid. Conditions for preparing chromatograms are given in text. The numbers inside the figure are percentage reflectance of the most dense part of each amino acid spot as measured at 550 millimicrons. Reflectance values between 90 and 100 tend to be unreliable.

The amino acid spots were revealed by dipping the dried chromatograms through a 0.2 per cent solution of ninhydrin in 95 per cent ethanol, followed by drying at room temperature and heating for 7 minutes at 95°. We have found that these conditions are best for inducing maximum color development and for reproducibility. It should be pointed out that a few amino acids, such as β-aminoisobutyric acid, will not react with

ninhydrin unless the chromatogram is heated. The amount of each amino acid was estimated by measuring the reflectance of spots on the chromatograms by means of a reflectance attachment to the Beckman Model B spectrophotometer. Standard curves were prepared by running known amounts of amino acids under identical procedures. Under these conditions, the per cent reflectance is usually proportional to the log of the concentration, as shown in Fig. 1.

Phenylthiocarbamide (PTC) taste sensitivity was measured by testing the ability of the subjects to taste serial dilutions of a 0.001 molar solution. The strongest solution (0.001 M) was designated concentration 1, 0.0005 M was designated 2, 0.00025 as 3, etc. The subjects were tested starting with the highest dilution (solution 9). Each subject was given a small bit of the test solution in a plastic spoon and was asked to report his reaction. If he failed to report a bitter taste, the next highest concentration was tested. When a subject reported a bitter taste, he was tested repeatedly with solutions in that region of concentration until the examiner was satisfied that the subject could consistently taste the PTC in one solution but not in the next higher dilution. At no time did the subject know which solution was being tested. The minimum concentration of PTC which could be tasted was recorded as the threshold for that subject. If the subject could not taste the strongest concentration, his threshold was recorded as 0 for purposes of statistical analysis.

RESULTS

Hematologic measures

The many measures which can be made on blood furnish examples of most of the problems of interpretation of hereditary versus environmental variability. All of the measures reported here are used extensively in clinical evaluation in a variety of diseases. This is ample evidence of the great amount of non-genetic variation which is possible. For this reason, many people would think of these measures as being relatively free of genetic control. However, such assumptions should not be made unless experimental evidence justifies them.

In this respect it is necessary to consider carefully what is meant by the genetic component of variation. In the present study, the basic comparison of variation is the within-pair variance of monozygotic twins versus the within-pair variance of dizygotic twins. The monozygotic pairs are assumed to be subject to all the sources of variation of the dizygotic pairs except genetic variation. Hence, the smaller the non-genetic variance, the greater the chances of demonstrating the action of genetic variation. For this reason, only normal twins have been studied in this sample. Thus the marked deviations which are of interest in clinical studies are absent in this series. Furthermore, only those twins have been studied which are still living together. This tends to minimize environmental variation and increase the relative contribution of genetic variation. The demonstration of a significant genetic variation does not, therefore, contradict the well-established fact that the non-genetic variation among hematologic measures is very large.

There have been few previous attempts to study genetic control of these measures. The two studies which are particularly relevant to the present report are those of Glatzel (1931) and Ostertag (1936). The design of both of these studies was the same as the present, although statistical analysis of the results was different. In both studies, hemoglobin levels, total red and white

cell counts, and differential white cell counts were determined, the results of the last being presented as percentages of the total white count. Considerable differences are found in the results of the two studies, however. For example, Ostertag found hemoglobin levels and red cell counts not to reflect genetic variation, while Glatzel found both these measures to show significant genetic variation. In the present study, results of which are presented in table 1, both these measures show genetic variation. In the case of hemoglobin, it can be seen that the genetic variation was contributed primarily by the males. This is not unexpected since females should be more subject to environmental variations in iron supply, etc. The variance ratio for the number of red blood cells is significant at the 5 per cent level when both sexes are pooled, but it is not significant when the sexes are analyzed separately, because of the smaller numbers of individuals. The same observation would appear to be true in our results in the case of white cells. Both Glatzel and Ostertag failed to find a significant genetic component in the determination of the total white cell count.

The hematocrit was not studied by the previous authors. In this study, variation in hematocrit does not show a significant variance ratio, nor do the derived values based upon cell volume. Nevertheless, the mean corpuscular hemoglobin, expressed as amount per cell, shows a significant variance ratio, again the genetic variation being due largely to the contribution of the males. Sedimentation rate does not show a significant genetic component when sexes are pooled, but the males do show a significant component.

In the two studies referred to above, differential white counts were reported, but only as per cent of the white count. Glatzel reported a significant genetic variation in the neutrophils, monocytes, and lymphocytes, the evidence in the case of the last being very weak. Basophils and eosinophils did not show significant genetic variation. Ostertag failed to find genetic variation in any of the differential counts. He explained the difference in his results as due to the age of the subjects, his being adults while Glatzel's were younger individuals.

The results presented in table 1 show that some of the variability in frequency of certain white cell types is heritable, however. Part of the difference between these results and others may be due to the fact that the values in table 1 are expressed as circulating cells per cubic millimeter rather than per cent of white cells. Of particular interest are the variations in numbers of eosinophils, which show significant variance ratios both with sexes pooled and for each sex separately. There have been a number of reports of elevated eosinophil counts in certain families, although it has not been possible to establish clearly the source of this increase (Neel, 1953). The present results would suggest that such individuals may represent the extreme of genetic variability. The values obtained for the eosinophils probably are more accurate than for other morphologic types, since they were counted directly. This may in part account for the more consistent results with eosinophils. In the case of basophils, the very large error associated with the small number of such cells encountered in a differential count would tend to obscure any genetic variation which might exist. The strongest evidence for genetic factors among the remaining morphological types is found in the polymorphonuclear neutrophils, where the genetic variation is contributed largely by females. Both lymphocytes and monocytes show

TABLE 1. ANALYSIS OF HEMATOLOGIC MEASURES OF TWINS. SUPERSCRIPTS INDICATE LEVEL OF SIGNIFICANCE
(N MZ♀ = 19 pr, MZ♂ = 23 pr, DZ♀ = 18 pr, DZ♂ = 13 pr)

Measure	$\bar{x}$♀	s♀	$\bar{x}$♂	s♂	t (♀-♂)	$\frac{S^2w\ DZ}{S^2w\ MZ}$	$\frac{S^2B\ MZ}{S^2w\ MZ}$	$\frac{S^2w\ MZ♀}{S^2w\ MZ♂}$	$\frac{S^2w\ DZ♀}{S^2w\ DZ♂}$	$\frac{S^2w\ DZ♀}{S^2w\ DZ♀}$	$\frac{S^2w\ DZ♂}{S^2w\ MZ♂}$
Red blood cells, 10^6/mm.^{3}	4.60	0.43	5.05	0.50	-5.87[3]	1.76[1]	7.40[2]	0.73	0.79	1.92	1.79
White blood cells, 10^3/mm.^{3}	5.79	1.32	5.87	1.10	-0.40	2.36[2]	3.32[2]	1.91	2.09	2.27[1]	2.08
Hemoglobin, Gm./100 ml.	13.19	1.19	14.70	1.56	-6.51[3]	3.23[2]	4.80[2]	0.81	0.32[1]	1.96	5.00[2]
Hematocrit, %	38.36	2.67	41.92	2.25	-7.89[3]	1.43	4.74[2]	0.50	0.46	1.47	1.59
Mean corpuscular vol,uμ^3	83.5	8.1	84.0	7.3	-0.34	0.92	7.03[2]	0.98	0.61	0.71	1.15
Mean corp. Hb, % of cell	34.5	2.0	35.1	2.8	-1.29	1.44	1.55	0.58	0.60	1.56	1.52
Mean corp. Hb, uμg/cell	28.8	3.5	29.4	3.9	-0.89	3.18[2]	4.24[2]	1.14	0.37[1]	1.75	5.56[2]
Sedimentation rate, mm./hr.	11.50	6.22	4.97	5.22	6.22[3]	1.47	6.90[2]	2.28	0.31[1]	0.49	3.57[2]
Eosinophils /mm^3	183.6	114.0	223.9	157.8	-1.76	2.62[2]	6.43[2]	0.66	0.47	2.33[1]	3.23[2]
Polymorphonuclear /mm.^{3}	3505	1127	3287	715	1.39	3.22[2]	2.24[2]	1.44	5.70[2]	4.17[2]	1.04
Basophils /mm.^{3}	182	187	253	263	-1.85	1.12	1.64	1.08	0.22[2]	0.44	2.13
Lymphocytes /mm.^{3}	1865	440	2014	473	-1.95	1.68	3.30[2]	1.96	1.18	1.33	2.22[1]
Monocytes /mm.^{3}	2681	1031	2704	1028	-0.14	1.88[1]	2.87[2]	0.83	1.47	2.44[1]	1.37

[1]P<0.05 [2]P<0.01 [3]P<0.001

significant heritability at the 5 per cent level, but this low level of significance requires that additional studies be made before definite conclusions can be drawn.

Serum constituents

The values for serum amino acid levels and creatinine are given in table 2. In the case of the amino acids, means and standard deviations have been expressed as percent reflectance of the amino acid spot resulting from the test amount of serum (25μl.). These values are related logarithmically to the absolute levels of amino acids, as shown in Fig. 1. Examination of the serum and urine levels of amino acids indicated that the percent reflectance is more nearly normally distributed than are the absolute values. Consequently, it was decided that for purposes of statistical analysis, the raw data would be superior.

Although amino acids have been studied in blood by a variety of methods, the authors are unaware of any studies designed to elucidate the role of genetic variation in differences in serum levels. Examination of table 2 will indicate that there is indeed very little variation in blood levels of most amino acids. There is an interesting variation between males and females in the levels of leucine. That there is a considerable interfamilial variation is indicated by the ratio of the between variance to the within variance for monozygotic twins. All but two of these ratios are significant, and those two are greater than 1.0. On the other hand, comparison of the within dizygotic variance to the within monozygotic variance failed to produce any significant ratios. Indeed the values fluctuate around 1.0, suggesting no tendency at all for genetic factors. The within monozygotic female variance divided by the within monozygotic male variance produced several significant ratios, all of the ratios being greater than 1.0. This suggests that females are more subject to environmental influences in blood amino acid levels. This trend failed to persist among dizygotic twins. The significant ratio obtained for the within dizygotic male variance compared to the within monozygotic male variance for arginine should probably be attributed to random fluctuation.

In the case of creatinine, the well-known sex difference is seen to be present. Here also, although there is significant resemblance between monozygotic co-twins, there does not appear to be a genetic component involved.

Saliva amino acids, pH, and PTC sensitivity

As can be seen from table 3, amino acid levels in saliva are appreciably lower in most cases than in blood. Furthermore, the distribution of amino acids is different. For example, glutamic acid and glycine form spots of approximately equal intensity. Of particular interest is spot 26. This substance has been designated by some as δ-amino valeric acid, although the substance has not been isolated and positively identified from saliva. It is a substance which is not seen in blood or urine, yet it occurs at much higher levels in saliva than do any other amino acids.

All of the "t" tests for differences in sex are positive, indicating that amino acids are more concentrated in saliva of males. Only the variations in aspartic acid, alanine and leucine are individually significant, however. It should be

TABLE 2. ANALYSIS OF SERUM LEVELS OF AMINO ACIDS AND CREATININE. SUPERSCRIPTS INDICATE LEVEL OF SIGNIFICANCE (N HZ♀ = 15 pr, N MZ♂ = 20, N DZ♀ = N DZ♂ = 13.)

Measure	$\bar{x}$ ♀	s ♀	$\bar{x}$ ♂	s ♂	t (♀-♂)	$\frac{S^2W^{DZ}}{S^2W^{MZ}}$	$\frac{S^2B^{MZ}}{S^2W^{MZ}}$	$\frac{S^2W^{MZ♀}}{S^2W^{MZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{DZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{MZ♀}}$	$\frac{S^2W^{DZ♂}}{S^2W^{MZ♂}}$
Aspartic acid, %R (25 μl)	97.31	1.52	97.23	1.30	0.30	1.22	1.42	1.09	0.90	1.10	1.33
Glutamic acid, %R (25μl)	89.65	4.49	88.45	2.11	1.78	1.62	4.23[2]	1.05	0.59	1.15	2.04
Glycine, % R (25μl)	88.75	2.80	88.09	2.19	1.40	0.49	1.44	1.41	0.96	0.40	0.58
Threonine, %R (25μl)	96.13	2.10	96.18	1.41	-0.14	1.05	1.91[1]	2.18	7.01[2]	1.35	0.42
Alanine, %R (25μl)	76.17	4.25	76.41	3.38	-0.33	1.05	2.70[2]	2.22[1]	0.97	0.71	1.64
Glutamine, %R (25μl)	77.04	3.31	76.42	3.00	1.04	1.00	3.08[2]	1.40	0.89	0.79	1.23
Valine, %R (25μl)	84.21	4.11	83.42	3.54	1.10	0.93	2.64[2]	2.46[1]	1.10	0.65	1.45
Leucine, %R (25μl)	90.35	3.68	88.33	2.42	3.41[3]	0.94	1.83[1]	5.74[2]	2.30	0.72	1.82
Arginine, %R (25μl)	93.71	2.55	92.95	1.91	1.78	0.79	1.94[1]	7.01[2]	1.27	0.46	2.56[1]
Creatinine, mg./ml.	.00823	.00175	.00963	.00171	-3.97[3]	0.67	4.03[2]	3.13[1]	1.12	0.47	1.32

[1]P<0.05 [2]P<0.01 [3]P<0.001

TABLE 3. SALIVARY AMINO ACID LEVELS, pH, AND PTC TASTE SENSITIVITY. PTC UNITS ARE AS GIVEN IN TEXT. SUPERSCRIPTS INDICATE LEVEL OF SIGNIFICANCE

(For amino acids, N MZ♀ = 10 pr. N MZ♂ = 8, N DZ♀ 11, N DZ♂ = 6; for pH and PTC, N MZ♀ = 20, N MZ♂ = 24, N DZ♀ = 22, N DZ♂ = 15.)

Measure	$\bar{x}$ ♀	s ♀	$\bar{x}$ ♂	s ♂	t (♀-♂)	$\frac{S^2W^{DZ}}{S^2W^{MZ}}$	$\frac{S^2B^{MZ}}{S^2W^{MZ}}$	$\frac{S^2W^{MZ♀}}{S^2W^{MZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{DZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{MZ♀}}$	$\frac{S^2W^{DZ♂}}{S^2W^{MZ♂}}$
Glutamic acid, %R (0.1 ml)	81.52	10.16	76.86	10.34	1.86	0.91	3.65[2]	1.03	0.75	0.81	1.11
Aspartic acid, %R (0.1 ml)	93.40	3.15	90.85	4.71	2.43[1]	0.91	1.35	0.12[2]	0.28[1]	2.08	0.90
Glycine, %R (0.1 ml)	81.10	9.40	79.00	11.29	0.81	1.75	4.19[2]	1.26	0.49	1.16	3.03
Alanine, %R (0.1 ml)	89.02	5.23	85.46	7.60	2.16[1]	1.22	3.19[2]	0.32[1]	0.97	2.33	0.78
Glutamine, %R (0.1 ml)	95.60	3.16	93.71	4.55	1.90	2.11	1.80	0.81	0.34	1.39	3.33
Valine, %R (0.1 ml)	96.45	3.64	95.15	4.99	1.15	1.32	2.81[1]	0.19[2]	3.65	5.00[2]	0.26
Leucine, % (0.1 ml)	96.71	4.19	94.27	4.79	2.14[1]	1.19	1.32	0.17[2]	4.20[1]	5.00[2]	0.20
Tyrosine, %R (0.1 ml)	96.95	3.34	95.92	4.92	0.94	0.85	1.02	0.41	0.44	1.00	0.93
Lysine, %R (0.1 ml)	93.19	7.87	91.68	9.31	0.71	0.77	1.87	2.63	0.33	0.33	2.63
Spot 26, %R (0.1 ml)	58.00	12.91	54.00	12.23	1.28	1.14	3.51[2]	0.75	4.20[1]	1.79	0.32
pH	6.98	0.26	7.04	0.29	-1.38	1.06	3.65[2]	0.96	0.77	0.98	1.22
PTC sensitivity	4.52	2.99	4.40	2.85	0.14	2.34[2]	5.87[2]	1.18	1.07	2.21[1]	2.43[1]

[1]P<0.05 [2]P<0.01 [3]P<0.001

noted that the number of twin pairs represented in this table are relatively small for amino acids and hence large differences must exist for significance to be demonstrable. There is also seen to be a considerable amount of co-twin similarity, but comparison of the dizygotic with monozygotic variances fails to indicate a strong genetic component in this variability. The two significant variance ratios for within dizygotic females to within monozygotic females would appear to be related to an unusually small within-pair monozygotic female variance.

The variation in ability to taste phenylthiocarbamide (PTC) is a well-known genetic trait, the ability to taste usually being considered to result from a single dominant gene. The distribution of tasting ability is clearly bimodal in a population, about 30 per cent of Caucasians being non-tasters. Similar results were obtained among the twin population reported here. A discussion of taste sensitivity can be found in Harris (1953). Although somewhat different concentrations were used for testing subjects in the present study, the distribution matches that reported by Harris, exactly.

Since PTC sensitivity is essentially a discontinuous trait, it is interesting to compare the results obtained from an analysis of variance to those obtained from separate classification into taster or non-taster. As seen in table 3, the resemblance of co-twins is very high and the comparison of within-pair variances of dizygotic and monozygotic twins is significant at the 1 per cent level. Furthermore, the variation within sexes is similar in all cases while significant heritability can be demonstrated taking either sex separately. On the other hand, if one classifies twins into concordant or discordant for PTC sensitivity, the results fail to differentiate between monozygotic and dizygotic twins. Of the 44 monozygotic twin pairs, 6 were clearly discordant for ability to taste PTC, while 11 of the 37 pairs of dizygotic twins were discordant. The chi-square just fails to be significant at the 5 per cent level.

Since PTC sensitivity is generally considered to be controlled by a single locus, it is surprising that any discordant pairs are to be found among twins considered to be monozygotic. However, several other authors have reported discordance among monozygotic twin pairs (Ardashnikov *et al.*, 1936; Rife, 1938; Verkade *et al.*, 1959). Furthermore, several matings between non-taster parents have been recorded in which taster children were produced. It thus seems inescapable that there are factors other than the one genetic locus which influence the ability to taste PTC. In several of the monozygotic twin pairs the differences in thresholds were several dilutions; thus the discordance does not always arise from a pair falling on either side of the dividing line of 0.00025 molar but still close to each other. Our results reemphasize the caution which must be exercised in using PTC sensitivity as a genetic marker. These findings are particularly relevant in view of the recent interest in the association between PTC taste sensitivity and thyroid disorders.

The salivary pH, although it also showed significant variation among twin pairs, failed to indicate a contribution of genetic variation to the variability.

Urinary amino acids

Since the introduction of paper chromatography, great interest has centered

around the reported variation among individuals in amino acid excretion in urine (Williams *et al.*, 1951). In certain diseases, such as galactosemia and Fanconi's renal rickets, the excretion of amino acids is grossly increased and readily detectable by paper chromatography. Among normal individuals, the existence of significant variation has been more difficult to demonstrate. Certain environmental factors are known to influence amino acid excretion to a limited extent. Most notable perhaps would be the excretion of methylhistidine following ingestion of anserine (Datta and Harris, 1951). Adrenal stimulation also can cause an increase in amino acids (e.g., Bergenstal *et al.*, 1951). Diet, at least a normal range of diets, does not appear to have a great influence on amino acid excretion, although it has not been possible to relate long term dietary habits to excretion. On the other hand, many authors have suggested that the major portion of variation is a function of the genetic makeup of the individual.

Perhaps the most relevant study previously done in this regard is that reported by Berry *et al.* (1955), and Gartler *et al.* (1955). These authors, using techniques essentially the same as those of this report, studied a series of adult twins, some of whom were living together and some of whom were living apart. They found the excretion of threonine, β-aminoisobutyric acid, lysine, and valine to be influenced by genetic variation significant at the 1 per cent level, while alanine, glycine, taurine, and tyrosine were significant at the 5 per cent level. Thus most of the amino acids would appear to show significant genetic variation. A greater intra-pair variance for dizygotic twins living apart was found compared to dizygotic twins living together, although this differential was not found in monozygotic twins.

It is interesting to compare their results with the data presented in this report. From the point of view of normal excretion levels, one interesting finding, confirming previous observations (Berry, 1953), is the higher excretion of glycine by females. It would appear that lysine is also generally excreted by females at increased levels compared to males.

It will be recalled that three samples were collected from each subject, the first being collected in the laboratory under carefully timed conditions, while the others were first morning samples. It is possible to analyze these three samples either separately or combined into an average value. We have chosen to use both of these procedures, the figure designated as mean representing the average of the three values for a single individual. Thus while samples 2 and 3 should be strictly comparable, sample 1 could conceivably deviate somewhat. Inspection of the population means for samples 1 compared to samples 2 and 3 suggest that very small deviations do indeed exist.

As a check of the reliability of the technical aspects of amino acid chromatography, two μg of α-aminobutyric acid were added to each chromatogram after the urine sample had been added. Analysis of the results reveals some very interesting facts. The standard deviation of the reflectance readings of this spot are the lowest of any amino acid spot, as it should be. However, the standard deviation of valine is very nearly the same as that for α-aminobutyric acid. One may use the variation of α-aminobutyric acid, then, as a standard against which to measure variation of other amino acids. It is interesting to

note, however, that if one analyzes α-aminobutyric acid for genetic factors and for intra-pair variability, significent results are obtained. In the comparison of between monozygotic variance to within monozygotic variance, three of the four ratios are significant at the one per cent level, while the fourth is significant at the five per cent level. Thus there appear to be rather strong influences in the urine sample on the reflectance values obtained for amino acids. Furthermore, if one looks at the within variance ratios, it would appear that in sample 1 collected in the laboratory there are rather strong influences which are themselves influenced by genetic variation. The nature of these forces can only be guessed at. It seems likely that the salt concentration of the urine is one of the major factors. This is somewhat surprising since α-aminobutyric acid migrates rather far away from salt. Nevertheless, the presence of high concentrations of salt may tend to cause the α-aminobutyric acid spot to be somewhat more diffuse. Variability in salt concentrations could well be more readily expressed in a mid-morning urine sample than in a first morning urine sample.

All of the amino acids appear to show a much greater similarity between co-twins than among the population as a whole. In nearly all cases, also, the variance ratio for within dizygotic to within monozygotic twin pairs is greater than one, suggesting genetic influence even though most of these do not attain significance. Perhaps the most interesting amino acid from the point of view of heritability is lysine, for which all four samples are significant. If males and females are separated, it can be seen that this heritability is due largely to the contribution of the females. On the other hand, β-aminoisobutyric acid, whose rate of excretion is a well-established genetic trait, shows a significant genetic component in only two of the samples. The explanation for this can probably be found in the fact that among this group of predominately Caucasian twins, the number of individuals who excreted appreciable amounts of β-aminoisobutyric acid was extremely small. It is very likely that a comparable study done in a population of Orientals would show a very high genetic component of variability (Sutton and Clark, 1955).

Among the ninhydrin-positive compounds which appear not to be influenced by genetic variations are ethanolamine, serine, taurine, and valine. This selection is somewhat arbitrary, since, with the exception of ethanolamine, all show significant heritability for at least one sample.

In table 5 are presented some additional measurements made on urine—specific gravity, pH, creatinine, and optical density. The three samples recorded in this table are comparable to the same samples reported in table 4, although in this case the mean values have not been separately analyzed. Specific gravity presumably is largely a measure of the manner in which the renal tubules handle water reabsorption. It is notoriously variable with environmental influences. Nevertheless, under standard conditions one might expect some genetic influence to be expressed in concentrating ability. In one small series of twins reported by Doxiades and Uhse (1934), monozygotic twins were reported to be more similar to each other in both urine specific gravity and pH than were dizygotic twins. The data were not given in their report. In a recent study by Aschner and Gartler (1959), twins were given a water load to see whether identical twins resemble each other more than do fraternal twins in the way in

TABLE 4. ANALYSIS OF URINARY AMINO ACID EXCRETION. VALUES GIVEN ARE PER CENT REFLECTANCE OF AN ALIQUOT OF URINE CONTAINING 50 μg CREATINE, WITH THE EXCEPTION OF α-AMINOBUTYRIC ACID WHICH WAS ADDED (2μg) TO THE CHROMATOGRAM. SUPERSCRIPTS ARE LEVELS OF SIGNIFICANCE. [N MZ♀ = 20 pr, N MZ♂ = 24, N DZ♀ = 21, N DZ♂ = 13, except for serine where N's = 6. 12, 8, 4 (sample 1); 11, 13, 10, 7 (sample 2); 12, 10, 12, 9 (sample 3); and 14 15, 15, 10 (mean) respectively.]

Measure	Sample	$\bar{x}$♀	s♀	$\bar{x}$♂	s♂	t (♀-♂)	$\frac{S^2W^{DZ}}{S^2W^{MZ}}$	$\frac{S^2B^{MZ}}{S^2W^{MZ}}$	$\frac{S^2W^{MZ♀}}{S^2W^{MZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{DZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{MZ♀}}$	$\frac{S^2W^{DZ♂}}{S^2W^{MZ♂}}$
α-Aminobutyric Acid, % R(2gμ)	1	64.51	3.34	63.95	3.14	1.01	2.33[2]	3.45[2]	0.33[1]	4.04[2]	7.14[2]	0.55
	2	63.96	3.07	64.03	2.90	-0.14	1.46	1.94[1]	0.64	0.59	1.47	1.61
	3	63.51	2.84	64.13	2.97	-1.27	1.25	2.26[2]	0.83	1.71	1.69	0.82
	Mean	63.86	2.27	64.04	2.10	-0.52	1.32	2.47[2]	0.67	1.78	2.04	0.77
Glycine, % R(50μgCr)	1	56.63	8.02	59.88	7.47	-2.61[1]	3.87[2]	7.34[2]	0.49	2.11	7.69[2]	1.79
	2	54.87	6.10	61.51	7.35	-6.11[3]	1.04	2.51[2]	1.36	0.91	0.86	1.28
	3	55.56	7.34	60.65	6.53	-4.58[3]	1.08	2.83[2]	1.62	0.58	0.67	1.89
	Mean	55.65	6.03	60.73	5.99	-5.28[3]	2.82[2]	6.01[2]	1.83	1.21	2.27[1]	3.45[2]
Alanine, % R (50μgCr)	1	69.34	8.51	67.31	7.11	1.62	1.93[1]	6.14[2]	1.19	4.06[2]	2.50[1]	0.73
	2	70.59	6.75	71.18	7.31	-0.52	0.89	3.98[2]	0.47[1]	0.49	1.02	0.99
	3	70.50	6.78	70.53	7.31	-0.02	1.00	2.54[2]	0.95	0.27[2]	0.51	1.82
	Mean	70.37	6.08	69.53	5.42	0.91	2.09[1]	5.09[2]	0.75	1.14	2.63[1]	1.72
Glutamine, % R (50μgCr)	1	61.06	7.89	58.88	7.59	1.76	1.86[1]	6.44[2]	0.56	1.37	3.03[2]	1.22
	2	63.05	6.98	62.51	8.33	0.43	1.06	6.38[2]	0.80	0.52	0.93	1.43
	3	62.76	7.23	62.34	7.78	0.35	1.15	3.05[2]	1.13	0.35[1]	0.64	2.04
	Mean	62.38	6.14	61.07	6.06	1.34	1.89[1]	6.53[2]	0.79	0.64	1.79	2.22[1]
Valine, % R (50μgCr)	1	90.39	2.93	91.83	4.65	-2.27[1]	0.66	4.05[2]	0.39[1]	0.42[1]	0.79	0.73
	2	92.15	3.23	92.19	3.63	-0.08	0.98	2.09[2]	0.96	1.51	1.15	0.73
	3	91.74	3.52	92.70	3.36	-1.71	3.32[2]	7.59[2]	0.72	0.58	3.23[2]	4.00[2]
	Mean	91.34	2.55	92.14	2.89	-1.81	1.12	3.37[2]	0.39[1]	0.98	2.04	0.82

[1]P<0.05 [2]P<0.01 [3]P<0.001

Leucine % R (50μgCr)	1	89.49	4.56	90.24	4.71	-1.00	1.06	7.01[2]	0.43[1]	0.88	1.71	0.85
	2	90.15	3.98	90.04	4.96	0.14	1.70	7.58[2]	0.97	0.23[2]	0.77	3.18[2]
	3	90.74	3.67	90.75	4.31	-0.01	1.26	4.93[2]	0.72	0.58	1.19	1.48
	Mean	89.96	3.70	90.09	4.15	-0.21	1.39	10.29[2]	1.00	0.43[1]	0.93	2.13
Tyrosine, % R (50μgCr)	1	88.94	4.26	87.49	5.16	1.89	2.36[2]	10.08[2]	0.47[1]	0.78	3.39[2]	2.07
	2	90.34	4.25	89.39	4.91	1.28	1.02	7.41[2]	0.69	1.93	1.54	0.56
	3	90.05	4.25	89.99	4.90	0.09	1.62	9.53[2]	0.82	0.25[2]	0.85	2.71[1]
	Mean	89.77	3.81	88.84	4.45	1.40	2.01[1]	13.36[2]	0.32[2]	0.46	3.03[2]	2.08
Lysine, % R (50μgCr)	1	86.83	10.44	89.50	8.74	-1.74	1.86[1]	2.89[2]	0.79	2.17	2.70[1]	0.99
	2	83.78	10.38	87.03	8.15	-2.18[1]	2.29[2]	5.31[2]	0.74	2.51[1]	3.57[2]	1.05
	3	84.50	9.66	86.95	8.12	-1.72	2.88[2]	3.51[2]	0.81	0.55	2.50[1]	3.70[2]
	Mean	85.07	8.50	87.68	6.32	-2.18[1]	3.66[2]	5.69[2]	0.37[1]	1.59	8.33[2]	1.92
Taurine, % R (50μgCr)	1	83.83	8.06	79.74	10.06	2.73[2]	2.23[2]	8.66[2]	1.60	0.75	1.61	3.45[2]
	2	80.02	9.83	80.65	8.16	-0.43	1.23	2.78[2]	1.21	1.18	1.19	1.22
	3	79.19	9.09	77.97	9.50	0.81	0.56	3.49[2]	1.55	0.98	0.44[1]	0.70
	Mean	81.01	7.11	79.18	6.53	1.68	1.32	5.49[2]	1.99	0.90	0.93	2.04
Serine, % R (50μgCr)	1	73.86	9.82	77.72	5.85	-1.82	1.13	2.83[1]	2.18	4.37	0.97	0.49
	2	76.48	5.17	76.93	5.73	-0.37	0.44	1.66	1.18	1.14	0.42	0.44
	3	75.56	5.93	76.95	5.30	-1.14	2.44[1]	6.02[2]	1.84	0.45	1.28	5.26
	Mean	75.00	5.81	76.62	4.76	-1.60	1.40	3.09[2]	2.24	0.78	0.90	2.63
Threonine, % R (50μgCr)	1	88.28	5.34	86.57	5.00	[2].04[1]	2.08[1]	4.96[2]	0.99	1.93	2.50[1]	1.30
	2	87.00	5.43	86.35	6.29	0.69	2.06[1]	2.85[2]	1.02	0.89	1.96	2.27[1]
	3	86.70	6.06	86.50	5.44	0.21	1.02	3.55[2]	2.40[1]	0.72	0.62	2.04
	Mean	87.38	4.47	86.34	4.21	1.50	3.24[2]	7.85[2]	1.04	1.17	3.45[2]	3.03[2]
B-Aminoisobutyric Acid, % R (50μgCr)	1	89.21	7.92	89.93	7.23	-0.57	0.56	4.70[2]	1.13	1.91	0.66	0.39[1]
	2	89.08	8.19	90.31	7.34	-0.95	1.74[1]	4.05[2]	1.33	2.90[1]	2.08	0.95
	3	89.33	8.34	91.19	6.99	-1.40	1.80[1]	6.36[2]	1.93	1.70	1.64	1.85
	Mean	88.79	7.24	90.57	6.08	-1.64	1.58	7.48[2]	1.22	2.22	1.85	1.01
Ethanolamine, % R (50μgCr)	1	91.85	7.27	91.41	7.73	0.37	0.87	3.19[2]	0.99	0.79	0.80	0.99
	2	91.41	6.23	90.32	7.64	0.96	0.64	2.27[2]	0.70	0.92	0.76	0.58
	3	91.34	6.13	89.22	6.83	2.01[1]	0.72	1.33	1.26	0.89	0.61	0.86
	Mean	91.54	4.66	90.04	5.89	1.75	0.59	3.42[2]	0.67	0.75	0.67	0.60

TABLE 5. MISCELLANEOUS URINE VALUES AND RENAL CLEARANCES. SUPERSCRIPTS ARE LEVELS OF SIGNIFICANCE. (For first 14 measures, N MZ♀ = 20 pr, N MZ♂ = 24, N DZ♀ = 21, N DZ♂ = 15; for creatinine clearance, N's = 14, 8, 8, 5 respectively; for urine/blood ratios, N's = 15, 20, 11, 11 respectively.)

Measure	Sample	$\bar{x}$ ♀	s ♀	$\bar{x}$ ♂	s ♂	t (♀-♂)	$\frac{S^2W^{DZ}}{S^2W^{MZ}}$	$\frac{S^2B^{MZ}}{S^2W^{MZ}}$	$\frac{S^2W^{MZ♀}}{S^2W^{MZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{DZ♂}}$	$\frac{S^2W^{DZ♀}}{S^2W^{MZ♀}}$	$\frac{S^2W^{DZ♂}}{S^2W^{MZ♂}}$
Specific gravity	1	1.0213	0.0064	1.0231	0.0069	-1.64	1.78[1]	4.77[2]	2.36[1]	1.30	1.38	2.50[1]
	2	1.0233	0.0067	1.0259	0.0058	-2.57[1]	1.85[1]	2.02[1]	6.07[2]	0.93	0.95	6.24[2]
	3	1.0238	0.0076	1.0250	0.0052	1.11	1.61	4.57[2]	1.54	5.59[2]	2.06	0.57
pH	1	6.130	0.744	6.030	0.813	0.81	1.19	4.95[2]	4.02[2]	2.62[1]	0.95	1.45
	2	5.957	0.535	5.829	0.396	1.70	0.58	1.16	0.90	2.34[1]	0.80	0.31
	3	5.891	0.490	5.860	0.403	0.436	2.12[1]	3.45[2]	0.35[2]	3.98[2]	6.21[2]	0.55
Creatinine, mg./ml.	1	1.25	0.60	1.50	0.66	-2.44[1]	2.43[2]	4.60[2]	1.47	2.41	2.63[1]	1.59
	2	1.69	0.74	1.94	0.80	-2.00[1]	1.78[1]	4.53[2]	1.38	2.25	1.92	1.18
	3	1.78	0.85	1.84	0.60	-0.44	1.34	2.12[2]	1.55	2.72[1]	1.42	0.82
Optical dens/Creatinine	1	39.46	14.33	37.93	13.37	0.68	0.86	6.88[2]	1.83	0.30[2]	0.33	2.00
	2	32.15	8.50	31.36	8.90	0.56	1.59	8.67[2]	3.57[2]	5.43[2]	1.41	0.93
	3	31.73	9.12	29.27	6.30	1.95	1.93[1]	3.65[2]	3.97[2]	1.13	1.20	4.35[2]
Urine, ml./min.	1	0.810	0.584	0.869	0.618	-0.61	1.38	5.95[2]	1.29	0.23[2]	0.51	2.85[1]
Urine, mg.creatinine/min.	1	0.733	0.277	1.028	0.299	-10.20[3]	1.72[1]	1.40	1.13	0.63	1.29	2.30[1]
Creatinine clearance, ml/min.	1	91.21	23.82	105.78	25.85	-2.78[2]	3.35[2]	4.36[2]	2.06	0.61	1.91	6.46[2]
Glycine, $\%R^{Bl} - \%R^{Ur}$		33.15	7.17	28.60	7.05	3.25[2]	3.24[2]	4.04[2]	0.28[2]	2.26	10.94[2]	1.38
Alanine, $\%R^{Bl} - \%R^{Ur}$		7.67	7.44	9.27	5.88	-1.27	2.56[2]	5.03[2]	0.53	4.65[2]	6.34[2]	0.72
Glutamine, $\%R^{Bl} - \%R^{Ur}$		16.00	7.14	17.77	7.18	-1.30	1.77	4.44[2]	0.63	1.72	2.99[1]	1.10
Valine, $\%R^{Bl} - \%R^{Ur}$		-6.10	4.53	-7.82	7.34	1.63	0.48	2.63[2]	0.53	0.79	0.64	0.43
Leucine, $\%R^{Bl} - \%R^{Ur}$		0.81	4.86	-1.77	4.95	2.79[2]	1.46	5.08[2]	0.92	1.25	1.71	1.25
Threonine, $\%R^{Bl} - \%R^{Ur}$		7.96	5.32	9.55	5.00	-1.61	2.58[2]	5.17[2]	1.51	1.17	2.24	2.90[1]

[1]P<0.05 [2]P<0.01 [3]P<0.001

which they were able to respond. The authors concluded that there was greater similarity in the water excretion curves of monozygotic twins than of dizygotic twins.

In the present study, there would also seem to be some evidence favoring a genetic component of variability. It will be noted that females have a slightly more dilute sample than the males, although both show a more concentrated sample collected in the laboratory than the first morning sample collected at home. There is a significant difference among twin pairs, and on the whole females would seem to be somewhat more variable, as indicated by the increased within variance.

It is interesting to compare the results on specific gravity with those on creatinine, expressed as milligram per milliliter. Creatinine excretion is generally considered to reflect largely the muscle mass of an individual. It is accordingly somewhat lower in females, when expressed on a 24 hour basis. Furthermore, since identical twins should have more nearly identical muscle mass, one would expect a genetic component in creatinine excretion when expressed on a 24 hours basis. However, when expressed in terms of milligrams per milliliter, the principal source of variability would ordinarily be the water excretion of the individual. Inspection of table 5 confirms that the genetic component of creatinine excretion does vary in a manner similar to specific gravity. The within female variance is uniformly greater than the corresponding within male variance, and dizygotic females would appear to be more variable than monozygotic females.

Another measure of water excretion is available for sample 1, that is, milliliters of urine formed per minute. In spite of a very large F ratio for the between versus within monozygotic variance, this measure fails to show a significant difference in within dizygotic versus within monozygotic variance. If males and females are treated separately, it can be seen that while the within monozygotic female variance is comparable to the within monozygotic male variance, the dizygotic female variance is much smaller than the within dizygotic male variance. This leads to a failure to show significant heritability among females, although a significant F ratio is obtained among males. Whether this sex difference is biologically significant cannot be established from these data.

The two variables, milligrams of creatinine per minute and creatinine clearance, should be relatively free of the urine dilution factor. Both of these measures show significant heritability, although creatinine clearance is the larger. If males and females are analyzed separately, it can be seen that the heritability component is contributed largely by males.

In the case of urine pH, there would seem to be little evidence of genetic variability. While it is true that sample 3 is significant at the 5 per cent level, inspection of the data reveals that this is contributed entirely by females. The explanation would appear to reside in a departure from normality which causes deviation of the variance.

Optical density of urine divided by creatinine concentration has been suggested as a measure of basal metabolic activity, at least if urine collection is made during basal periods (Vorzimer *et al.*, 1949). The measure has not been

extensively investigated and has not proved particularly useful for clinical purposes. The results in table 5 indicate that although there is a great deal of inter-twin pair variation, there do not appear to be important genetic factors involved.

Urine-blood ratios of amino acids

It was seen above that creatinine clearance showed a greater influence of genetic variation than did other expressions of creatinine excretion. One might expect the same to hold true for other urinary constituents. For six of the amino acids, there are data for levels both in urine and in blood. These can be used to calculate a quantity comparable to "clearance." As has already been pointed out, the reflectance values were used in analyzing data on blood and urine levels of amino acids, since these were more nearly normally distributed than were the absolute values. Since %R is a logarithmic function of the absolute concentration, one can obtain ratios of blood to urine levels simply by subtracting one from the other. The figure which emerges from such manipulations is in effect a transformation of the absolute ratio and should yield the same results statistically as do actual ratios.

The comparison of blood with urine values is given in table 5. Certain features of the distributions might first be noted. The high values of the glycine indicate that glycine is relatively concentrated in urine compared to other amino acids. Glycine also is an amino acid which is excreted at higher levels in females, and the ratio of blood to urine reflects this difference. On the other hand, valine is excreted at rather low levels in urine, considering the blood levels of valine. Thus the $\%\ R^{Bl} - \%\ R^{Ur}$ values are negative for valine. By comparing the within dizygous variance to the within monozygous variance, one obtains a significant ratio for glycine, alanine, and threonine. From table 4 it can be seen that of these six amino acids threonine is probably the one showing the greatest genetic component of variability. Both glycine and alanine show some genetic variation, but not significantly more than do glutamine, valine, or leucine. While it is difficult to give an objective statement on the relative significance of genetic variability in renal clearances versus blood and urine levels, there would seem to be some justification for the idea that renal clearances are somewhat more responsive to genetic variation.

DISCUSSION

A number of problems arise when one attempts to evaluate a study of this magnitude. Perhaps the question which occurs first is whether or not many of the results, both for those measures which do show significant heritability and those which fail to show significant heritability, are spurious because of sampling problems. Probably few if any of the values significant at the one per cent level are spurious. However, it seems highly likely that many of the values which are significant at the five per cent level are falsely significant and that many ratios which fail to show signficance should actually show significance.

Perhaps a more serious source of error for most of the comparisons arises from the lack of normality of the data. Only a few particularly aberrant pairs

in a population are sufficient to increase the variance greatly. This is a serious consideration in computation of within variance ratios, such as are used in this analysis. A discussion of the statistical problems involved in using twin data for estimates of heritability has been preented by Clark (1956).

An interesting suggestion presented by Gartler *et al.* (1955) to explain some of their findings was that identical twins tend to choose more similar environments than do fraternal twins. In the present study, all twins were still living together, in all but a few cases at home. Thus they had had relatively little opportunity to select their environments. This may be strictly true only at a superficial level, since an individual within a given set of surroundings may take advantage only of a limited part of his opportunities. It is not possible to throw light on this question in the present study. It seems unlikely, however, for the measures reported here that environmental differences within dizygotic twin pairs were sufficiently greater than for monozygotic pairs so that the apparent contribution of "genetic" factors was increased.

It is worth reemphasizing that the variability under scrutiny is the perhaps limited variability to be found within dizygotic twin pairs of predominantly Caucasian ancestry and similar environments. It is therefore treacherous to generalize from these results to all populations in all environments. The results do suggest that many of the variables which show a continuous distribution and which even are subject to environmental fluctuations are influenced, sometimes markedly, by genetic variation. The importance of such genetic variation in clinical medicine has yet to be ascertained.

SUMMARY

The twin method has been used to estimate the heritability of a number of biochemical and hematological measures. The subjects were 45 sets of monozygous twins and 37 sets of dizygous twins aged 12 to 20 years. Blood, urine, and saliva were collected for investigation of biochemical variables. Paper chromatography was used extensively for the estimation of amino acid levels. The within-monozygotic-twin-pair variance was compared to the within-dizygotic-twin-pair variance for each measure to obtain an estimate of the genetic component of variability. Tables are given which show these and other statistical parameters for each substance measured.

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