Genetic Epidemiology 2:17–27 (1985)

Genetic and Environmental Causes of Variation in Renal Tubular Handling of Sodium and Potassium: A Twin Study

J.B. Whitfield and N.G. Martin

Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown (J.B.W.) and Department of Population Biology, Research School of Biological Sciences, Australian National University, Canberra (N.G.M.), Australia

We have conducted a study of renal sodium and potassium reabsorption in 205 pairs of twins on freely chosen diets; 89 of the subjects were studied on more than one occasion. Renal tubular sodium and potassium handling, as measured by the fractional excretions FE_{Na} and FE_K, show repeatable differences between individuals.

Siblings (in this case monozygotic and dizygotic pairs of twins) are more alike in this respect than unrelated individuals. Comparison of monozygotic and dizygotic twin pairs indicates that genetic, rather than shared environmental, factors are probably responsible for this similarity, with heritability estimates of 0.5 for sodium and 0.6 for potassium. There are indications of sex differences in the sizes of the genetic and environmental effects for both variables and indications that the genetic effects may be qualitatively different for FE_K. Such findings need further investigation.

Key words: sodium, potassium, cation excretion, twins

INTRODUCTION

Interest in sodium metabolism, especially sodium transport and sodium intake, has been stimulated by theories that link high sodium intake with the development of essential hypertension [Dahl, 1972; Tobian, 1979; Morgan et al, 1979; de Wardener

Received for publication August 21, 1984; revision received November 6, 1984.

N.G. Martin's present address is Department of Human Genetics, Medical College of Virginia, P.O. Box 33, Richmond, VA 23298.

Address reprint requests to Dr. J.B. Whitfield, Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia.

and MacGregor, 1982; Simpson, 1979]. The factors that regulate human sodium intake or handling may therefore be of clinical as well as physiological importance. Since much of the epidemiological evidence might equally well be explained by low potassium intake as by high sodium intake [Bulpitt, 1981], and since both sodium restriction and potassium supplementation can produce a decrease in blood pressure (MacGregor et al, 1982a,b], it is possible that potassium may be just as important as sodium in the processes leading to hypertension.

Factors influencing an attribute such as sodium or potassium intake or excretion may be divided into those that are genetic, those environmental factors that are shared by members of a family but vary between families, and those that affect an individual independently of his familial environment. The relative contributions of these can be evaluated by twin studies, and if it is found that one of them is the major determinant of the repeatable differences between individuals this can point the way to further experimental or epidemiological studies.

Many studies have been carried out on 24-hr urine collections, but as they are highly variable from day to day, difficult to organize and sometimes inaccurate, and have the risk that knowledge of the impending collection will alter the subjects' activities, diet, or fluid consumption, there are advantages in alternative approaches. Apart from physiological relevance, the appropriate criteria for measures of intake, excretion, or metabolism are ease of collection, noninterference with the subjects' usual habits, and repeatability between occasions.

We have chosen to study renal tubular sodium and potassium handling by calculating the fractional excretion, which is the proportion of the sodium or potassium presented to the tubules that is allowed to pass through into the urine. This is calculable from the ratio of sodium or potassium clearance to the creatinine clearance on an untimed urine specimen, and it is a variable that can be seen to have a physiological meaning in its own right. The degree of reabsorption of sodium or potassium will ultimately depend on the balance between intake and nonrenal losses.

We have studied the constancy of the tubular fractional excretions in twins of both sexes who attended on more than one occasion at intervals ranging from 1 to 17 months, and we have assessed genetic and familial influences on renal tubular cation handling by comparison of the similarities within monozygotic and dizygotic twin pairs.

SUBJECTS AND METHODS

Twins

Pairs of monozygotic (MZ) and dizygotic (DZ) twins, aged between 18 and 34 years (mean 23.1), were recruited from the Australian NH&MRC Twin Registry for a study of alcohol metabolism and susceptibility to intoxication [Martin et al, 1984]. Both members of a twin pair attended on the same day, and the study extended over all seasons. Eighty-nine individuals (50 men and 39 women) attended on more than one occasion and the results from these 89 are used to assess medium term repeatability of the measurements within an individual. The twins arrived at about 9 a.m., having had a light, nonfatty breakfast at about 8 a.m., and blood and a nontimed urine sample were collected soon after arrival and before any alcohol was ingested. Fifteen milliliters of venous blood, taken into a tube containing ammonium heparin,

was centrifuged within 2 hr of collection and analyzed for, inter alia, sodium, potassium, and creatinine.

All twins were blood typed with the following antisera; anti-A, A_1, B, C, c, D, E, e, M, N, S, s, Fy^a, K, and Jk^a and were typed for serum alpha-1-antitrypsin (Pi). Twins were diagnosed as DZ on the basis of difference in sex, at least one marker locus, or, in a few cases, large differences in height, coloring, or other morphological features. In remaining cases of doubtful zygosity several more genetic markers were typed. It is possible, however, that there are a few pairs diagnosed as MZ who on still further typing would prove to be DZ.

Of the 205 twin pairs for whom measurements were available, there were 42 MZ female, 42 MZ male, 44 DZ female, and 38 DZ male pairs and 39 DZ pairs of opposite sex (DZOS). There were no substantial differences in age distribution between the five zygosity groups.

Analytical Methods

Plasma sodium, potassium, and creatinine were measured on a Technicon SMAC by ion-selective electrodes and the Jaffe reaction. Urine sodium and potassium were measured with an IL343 flame photometer and urine creatinine by the Jaffe reaction on a Technicon SMA 6/60.

Calculations

The fractional excretions of sodium (Fe_{Na}) and potassium (FE_K) were calculated from the ratios of their clearances to creatinine clearance, eg, $(Na_u \times Cr_p) / (Cr_u \times Na_p)$ where u and p denote urine and plasma concentrations, respectively. Analysis of variance and calculation of the within- and between-individual or within- and between-pair components of variance were performed by standard methods. The intraclass correlation coefficient, used to assess the repeatability (test-retest reliability) of the various measurements within an individual is $S_B^2/(S_W^2 + S_B^2)$, the proportion of the total variance explained by differences between individuals. If each person is unique and constant, this value will be 1.0.

Fitting Models of Variation

The statistics to which models of variation are fitted are the between- and within-pair mean squares (WMS) from an analysis of variance of each separate group of n twin pairs. The degrees of freedom and expected mean squares between pairs and within pairs are $n - 1$, $S^2_w + 2S^2_b$ and n, S^2_w, respectively.

A large difference in means of males and females will inflate the WMS of DZ opposite sex (DZOS) pairs by an amount $n/2(\bar{M} - \bar{F})^2$ where there are n pairs. $\bar{M}$ is the male mean and $\bar{F}$ is the female mean. The DZOS WMS are thus corrected for this amount and the corresponding degrees of freedom removed.

Models of variation to explain these mean squares can now be fitted using the method of iterative weighted least squares, described extensively elsewhere [Martin, 1975; Eaves and Eysenck, 1975].

A simple model for variation in MZ and DZ mean squares is given by Clark et al, [1980]. The sources of variation are E_1, environmental variance within families, which is specific to the individual and will include error variance, and E_2, which on the other hand, includes sources of environmental variance shared by members of a

family but differing between families. E_2 will, thus, include the lasting effects of cultural and class differences and parental rearing practices. Here it may include dietary habits that both members of a twin pair share in common, but that differ between pairs. The third source of variation is V_A, which is that part of the genetic variation due to the additive effects of genes in the absence of assortative mating. The appropriateness of different models is tested by the chi square criterion. A model is only elaborated if a simpler one fails or a significant improvement is made by adding a further parameter.

A sensible hierarchy of models is to first fit E_1 alone. Failure of this most simple model will indicate that there is significant between-families variation. A model incorporating E_1 and E_2 will test whether the between-families variation is entirely environmental in origin, while the E_1V_A model will test whether it is entirely genetic. If both two-parameter models fail, then a model incorporating all three sources of variation must be considered.

There is no necessary reason why the components of variation will be the same in both males and females, so models are first fitted to the sexes separately and then to the eight statistics together. At this stage, a heterogeneity chi square for k df can be calculated by adding the two male and female chi squares for 4 − k df and subtracting from the chi square (8 − k df) for the corresponding model fitted to all eight statistics. The heterogeneity chi square for k df will indicate whether the same parameters are appropriate for both sexes. If it is not significant, then the DZOS data may be added and the same model fitted to all statistics.

RESULTS

Repeatability

The twins who returned for a second or, in six instances, a third visit did so at intervals ranging from 1 to 17 months (mean 4.5 months) after the first.

The analyses of variance for sodium and potassium, in men and women, are shown in Table I. The results were calculated on the raw data and after log-transformation; because the genetic analysis was performed on the log-transformed results (see below), the repeatabilities are shown after transformation to allow direct comparison.

TABLE I. Repeatability Results for the Subjects Who Attended on More Than One Occasion

		Raw scale	Log-transformed	Log-transformed[a]		
	df	F	F	S_W^2	S_B^2	R_i
FE_{Na}						
Male twins	49,54	2.42***	2.56***	0.036	0.028	0.44
Female twins	38,41	1.89*	2.74***	0.042	0.037	0.46
FE_K						
Male twins	48,53	1.71*	1.37 (NS)	0.046	0.009	0.16
Female twins	38,41	1.91*	2.41**	0.037	0.026	0.42

[a] S_W^2, within-person, and S_B^2, between-person components of variance; R_i, intraclass correlation coefficient.
*P < 0.05.
**P < 0.01.
***P < 0.001.

Calculation of the repeatability coefficients using the logarithmic scale reduced the weight given to high values and increased all the intraclass correlation coefficients except that for FE_K in males.

The relationship, if any, between length of time between visits and the magnitude of the difference in the results was calculated for men and women separately. No significant correlations were found.

Genotype-Environment Interaction and Scale

The correlation of individual MZ pair absolute differences (which can only be due to environmental effects) with their corresponding pair sums (a measure of genetic effects) is now well established as a test for one class of systematic genotype-environment interactions [Jinks and Fulker, 1970; Clark et al, 1980]. Such interactions may confound any model for the additive action of genetic and environmental effects but can usually be removed by an appropriate transformation of the scale of measurement.

This test was carried out for FE_{Na} and FE_K and large positive correlations were found in both male and female MZ twins, particularly for FE_K. Correlations were also found between absolute occasion differences and occasion sums in the individuals who were measured on two occasions.

These results indicate that the sources of variance are not uniform over the range of measurement and this is reflected in the significant positive skewness in the distribution of both variables. If this heteroscedasticity is not removed, any attempt to provide a global partitioning of the variance into genetic and environmental components will be meaningless since environmental effects are shown by the existence of the correlations to be more important relative to any genetic effects at the upper end than at the lower end of the raw scale of measurement.

However, transformation of the raw measurements to a logarithmic scale removed the distributional skewness and the correlations between pair sums and absolute differences in both variables. Clearly, it is appropriate to use this scale when we proceed to the genetic analysis.

Genetic Analysis

There is a significant difference both in mean and variance between males and females for log FE_{Na} but in neither for log FE_K. However, no significant differences in means and variances were found between MZ and DZ groups of a given sex. We may thus proceed to estimate the relative importance of genetic and environmental factors on variation in sodium and potassium fractional excretion [Jinks and Fulker, 1970].

Mean squares for FE_{Na} and FE_K, calculated on a $\log_{10}$ scale, are shown in Table II. The results of fitting models of variation to the female and male data separately, the male and female data combined (F + M), and to these data sets plus the opposite sex mean squares (F + M + OS) are shown in Table III for sodium and Table IV for potassium.

Both variables showed similar results. The E_1 model was strongly rejected in both sexes, demonstrating that there is significant variation between pairs of twins. However, either family environment or additive genetic variation could satisfactorily account for this between-pairs variation since neither the E_1E_2 nor the E_1V_A model is rejected at the 5% level of significance. Even so, the E_1V_A model was clearly more

TABLE II. Observed Mean Squares and Their Degrees of Freedom for Sodium and Potassium Fractional Excretion Measured on Log_{10} Scale

Mean squares	df	$\log_{10} FE_{Na}$	$\log_{10} FE_K$
MZ females			
Between	41	0.105297	0.097066
Within	42	0.035013	0.015232
MZ males			
Between	41	0.087613	0.068021
Within	42	0.030736	0.022685
DZ females			
Between	43	0.105366	0.078890
Within	44	0.060728	0.031971
DZ males			
Between	37	0.057521	0.049462
Within	38	0.035256	0.024076
DZ opposite-sex			
Between	38	0.117215	0.060239
Within	38	0.064290	0.049060

TABLE III. Results of Fitting Models of Variation to Results for Log_{10} Sodium Fractional Excretion

	$\hat{E}_1$	$\hat{E}_2$	$\hat{V}_A$	df	χ^2	h^2
Females						
E_1	0.0764***			3	13.11**	
E_1E_2	0.0482***	0.0286**		2	3.06	
E_1V_A	0.0365***		0.0409***	2	0.52	0.53 ± 0.10
$E_1E_2V_A$	0.0363***	−0.0019	0.0430	1	0.52	0.56 ± 0.33
Males						
E_1	0.0529***			3	14.79**	
E_1E_2	0.0329***	0.0202**		2	1.83	
E_1V_A	0.0286***		0.0240**	2	1.09	0.46 ± 0.11
$E_1E_2V_A$	0.0291***	0.0052	0.0184	1	1.01	0.35 ± 0.37
Females + males						
E_1	0.0651***			7	33.21***	
E_1E_2	0.0408***	0.0246***		6	10.95	
E_1V_A	0.0329***		0.0325***	6	6.69	0.50 ± 0.07
$E_1E_2V_A$	0.0330***	0.0013	0.0311*	5	6.69	0.48 ± 0.25
Females + males + opposite sex						
E_1	0.0699***			9	38.39***	
E_1E_2	0.0452***	0.0250***		8	14.66	
E_1V_A	0.0339***		0.0367***	8	9.48	0.52 ± 0.07
$E_1E_2V_A$	0.0339***	0.0004	0.0363*	7	9.48	0.51 ± 0.22

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

TABLE IV: Results of Fitting Models of Variation to Results for Log_{10} Potassum Fractional Excretion

	$\hat{E}_1$	$\hat{E}_2$	$\hat{V}_A$	df	X^2	h^2
Females						
E_1	0.0554***			3	30.43***	
E_1E_2	0.0238***	0.0320***		2	5.77	
E_1V_A	0.0148***		0.0403***	2	0.23	0.73 ± 0.06
$E_1E_2V_A$	0.0152***	0.0068	0.0337*	1	0.00	0.60 ± 0.26
Males						
E_1	0.0411***			3	17.07**	
E_1E_2	0.0234***	0.0179***		2	0.99	
E_1V_A	0.0206***		0.0201***	2	1.39	0.49 ± 0.11
$E_1E_2V_A$	0.0217***	0.0119	0.0075	1	0.72	0.18 ± 0.34
Females + males						
E_1	0.0485***			7	55.55***	
E_1E_2	0.0236***	0.0252***		6	9.71	
E^1V_A	0.0180***		0.0301***	6	7.09	0.63 ± 0.06
$E_1E_2V_A$	0.0187***	0.0097	0.0202*	5	5.88	0.42 ± 0.21
Females + males + opposite sex						
E_1	0.0497***			9	53.94***	
E_1E_2	0.0283***	0.0215***		8	21.43**	
E_1V_A	0.0191***		0.0308***	8	10.34	0.62 ± 0.06
$E_1E_2V_A$	0.0187***	−0.0016	0.0325**	7	10.20	0.65 ± 0.20

*P < 0.05.
**P < 0.01.
***P < 0.001.

appropriate in females, although in males there was little to choose between the two models. Significant heritability estimates, calculated as $h^2 = \hat{V}_A/\hat{V}_T$ where $\hat{V}_T = \hat{E}_1 + \hat{V}_A$ for the E_1V_A model, were obtained for the two parameter models in both sexes.

Because one needs large numbers of twins to reject an E_1E_2 model for traits of intermediate heritability, even when there is no E_2 effect [Martin et al, 1978], greater discrimination between models is achieved by combining the data sets. When the models were fitted to all 10 statistics (from all five zygosity groups) it was clear that the E_1V_Amodel is more appropriate than the E_1E_2 model and that no improvement in fit is gained by fitting all three parameters. Heritability estimates of 0.52 for log FE_{Na} and 0.62 for log FE_K resulted, as shown in Tables III and IV.

However, this procedure ignores heterogeneity in the sources of variation in the two sexes. The increase in chi square caused by combining the sexes for the E_1V_A model for log FE_{Na} was $\chi^2_2 = 5.08$ and for log FE_K was $\chi^2_2 = 5.47$. Neither heterogeneity is quite significant at the 5% level but there is clearly a strong indication that it may be misleading to attribute exactly the same causes of variation to males and females.

A full model incorporating different sized E_1, V_A and E_2 effects for males and females [Clark et al, 1980; Eaves et al, 1978] was therefore tried. If the genes affecting a trait in males are quite different from those affecting the trait in females, then $\hat{V}_{A_{mf}}$ (the covariance between the genetic effects in males and females) will be

zero. If the genes acting in males and females are the same but produce scalar differences in the two sexes, the correlation between the effects

$$r_{V_A} = \frac{V_{Amf}}{\sqrt{(V_{Am} \cdot V_{Af})}}$$

will be one. A similar argument applies to $E_{2_{mf}}$, the covariation between E_2 effects acting in males and females. If $\hat{V}_{A_{mf}}$ and $\hat{E}_{2_{mf}}$ are zero, the between- and within-pairs mean squares for DZOS pairs will be equal, ie, the intraclass correlation for DZOS pairs will be zero.

Fitting separate E_1 parameters for males and females produced little improvement in fit, but allowing different additive genetic estimates for the two sexes produced an improvement, particularly for log FE_K ($\chi^2_2 = 6.67$, $P < 0.05$). The results of fitting E_1V_A models with different V_A for males and females are shown in Table V. Different heritability estimates for the two sexes show higher proportions of the total owing to genetic factors in females for both variables. However, whereas for log FE_{Na} we found $r_{V_A} = 0.98$, for log FE_K, $r_{V_A} = 0.30$, indicating that the same genetic effects are operating in males and females for sodium excretion but that at least some of these factors may differ between the sexes for potassium excretion.

Because there was some hint from the preliminary model fitting that family environmental factors might be important in males but not in females, we also fitted a model including, in addition to the above, an E_2 parameter for males. These results are also shown in Table V and, although there was only a trivial reduction in chi square for the extra degree of freedom so that this model has a lower probability than the previous one, we now see that the between-families variance in males would be divided between E_2 and V_A and that a zero heritability would be compatible with the male but not the female data.

TABLE V. Results of Fitting Models With Different Sources of Variation in Males and Females

	Log_{10} FE_{Na}	Log_{10} FE_K
$E_1V_{A_m}V_{A_f}V_{A_{mf}}$ Model		
$\hat{E}_1$	0.0329**	0.0180**
$\hat{V}_{A_m}$	0.0241**	0.0228**
$\hat{V}_{A_f}$	0.0513**	0.0398**
$\hat{V}_{A_{mf}}$	0.0343*	0.0092
	$\chi^2_6 = 5.71$	$\chi^2_6 = 3.67$
	$h^2_m = 0.42 \pm 0.09$	$h^2_m = 0.56 \pm 0.08$
	$h^2_f = 0.61 \pm 0.07$	h^2_f ↕ 0.69 ± 0.06
$E_1E_{2m}V_{A_m}V_{A_f}V_{A_{mf}}$ Model		
$\hat{E}_1$	0.0334**	0.0184**
$\hat{E}_{2m}$	0.0107	0.0085
$\hat{V}_{A_m}$	0.0131	0.0142
$\hat{V}_{A_f}$	0.0507**	0.0394**
$\hat{V}_{A_{mf}}$	0.0344*	0.0093
	$\chi^2_5 = 5.47$	$\chi^2_5 = 3.21$
	$h^2_m = 0.23 \pm 0.33$	$h^2_m = 0.34 \pm 0.29$
	$h^2_f = 0.60 \pm 0.07$	$h^2_f = 0.68 \pm 0.06$

*$P < 0.05$.
**$P < 0.001$.

DISCUSSION

To study the heritability of any physiological character it is necessary to either assume or demonstrate that individuals differ from each other and that each individual remains relatively constant over time. The heritability of a trait should be less than or equal to its repeatability [Falconer, 1980]. Any theory that seeks to implicate such a character as a cause of a chronic disease process also implicitly makes these assumptions.

Many authors have shown that daily sodium or potassium output is variable from day to day and have calculated in various ways that about one third of the total observed variance is due to the differences between people and the remainder to variation within an individual (including analytical error) [Liu et al, 1979; Shephard et al, 1981]. Our repeatability results are similar to this and to our (unpublished) results on 24-hr output; the renal tubular sodium and potassium transport, measured in the midmorning, displays about the same balance between the within- and between-person components of the total variance. One exception to this is the value for potassium in the men, especially with the log-transformed results.

This being so, there must be factors that act to maintain a person at the higher or lower end of the scale; in general they could involve 1) familial environmental factors, such as acquisition of dietary preferences in infancy; 2) genetic factors, such as a genetic difference in salt appetite, hormonal control mechanisms, or the tubular transport proteins; or 3) nonfamilial environmental factors, unique to each individual but constant over time. These sources of variation can be resolved if certain assumptions are made, by studies on MZ and DZ twin pairs. The assumptions are first that twins are representative of the general population and second that similarities owing only to the shared external environment exist to the same extent in MZ and DZ pairs. These issues are discussed for a (probably) polygenic threshold condition by Kendler [1983].

Our results show, by the rejection of the E_1 model, that there are familial similarities for both sodium and potassium renal tubular transport. The next step is to consider whether they are genetic in origin or due to the persisting effects of shared environments.

In women, genetic factors account for most of the non-E_1 variation, for both sodium and potassium, with heritabilities of 0.61 ± 0.07 and 0.69 ± 0.06, respectively. In men, the presence of E_2 factors cannot be excluded, and if they exist the heritabilities could lie anywhere between 0.23 and 0.42 for log FE_{Na} and 0.34 and 0.56 for log FE_K. Whereas it appears that the genetical effects acting in males and females are the same for sodium excretion, for potassium at least some of the genetic factors acting may be different in the two sexes.

The fact that estimated heritabilities are higher than the calculated repeatabilities, particularly in females, is paradoxical. Certainly the repeatabilities are calculated on a very much smaller subset of the data than the heritabilities and sampling error could provide an explanation. In addition, the apparent repeatability could be lowered by climatic differences between the two occasions, usually some months apart, when the measurements were made. Any effects owing to genuine seasonal variation or to batch variation in the assay will be estimated as within-individual variation in the repeatability analysis and will tend to lower the repeatability estimates. Such effects would be estimated as between-pairs variation in the twin analysis and, while they should be estimated as E_2 effects, the notorious difficulty in separating E_2 and V_A in

classical twin studies [Martin et al, 1978] may lead to inflation of the heritability estimate. In any case, the finding that the repeatability is no greater than the heritability indicates that individual differences of an environmental nature do not contribute to the long-term position of each person in the overall range.

On balance, the results of this study show that there are genetic factors that can influence renal tubular sodium and potassium handling in normal humans on freely chosen diets, even if the effect is more detectable in women than men. The only similar study we are aware of [Grim et al, 1979] showed that genetic factors influenced renal sodium and potassium fractional excretion after a sodium load, but did not detect genetic influences on either before the load. This might in part be due to the smaller number of twin pairs in that study.

Our results do not, of course, show which of the many physiological processes involved in sodium and potassium balance are under genetic control, but several possibilities should be considered. The degree of reabsorption of sodium or potassium from the glomerular filtrate will be mainly influenced by the balance between intake and nonrenal losses. Therefore, genetic control of salt appetite leading to a high or low salt intake would be expected to result in greater or lesser tubular passage of sodium. In animal experiments, genetic differences in salt appetite have been demonstrated between strains of rats, probably mediated through brain angiotensin II [Dinicolantonio et al, 1982], but it is not possible to say whether similar mechanisms operate in humans.

Alternatively, there could be an intrinsic tendency in the renal tubules to excrete either more or less sodium [de Wardener and MacGregor, 1982] or potassium: this would have to lead to a change in appetite so that an equilibrium between output and intake is maintained. It appears that at least in some animals [Weisinger et al, 1979], appetite for salt can change in a way that tends to maintain homeostasis.

Whatever the cause, there is evidence that renal tubular sodium and potassium handling can show significant heritability in humans.

ACKNOWLEDGMENTS

We are grateful to the twins of the Australian NH&MRC Twin Registry who participated in this study. We thank Janet Craig, who organized collection of the twin samples, and the Australian Associated Brewers, who funded the larger project of which this was part.

REFERENCES

Bulpitt CJ (1981): Sodium excess or potassium lack as a cause of hypertension: A discussion paper. J R Soc Med 74:896–900.

Clark P, Jardine R, Martin NG, Stark AE, Walsh RJ 1980): Sex differences in the inheritance of some anthropometric characters in twins. Acta Genet Med Gemellol 29:171–192.

Dahl LK (1972): Salt and hypertension. Am J Clin Nutr 25:231–244.

de Wardener HE, MacGregor GA (1982): The natriuretic hormone and essential hypertension. Lancet i:1450–1454.

Dinicolantonio R, Hutchinson JS, Mendelsohn FAO (1982): Exaggerated salt appetite of spontaneously hypertensive rats is decreased by central angiotensin-converting enzyme blockade. Nature 298:846–848.

Eaves LJ, Eysenck HJ (1975): The nature of extraversion: A genetical analysis. J Pers Soc Psychiatry 32:102–112.

Eaves LJ, Last KA, Young PA, Martin NG (1978): Model-fitting approaches to the analysis of human behaviour. Heredity 41:249–320.

Falconer DS (1980): "Introduction to Quantitative Genetics," 2nd ed. London: Longman.

Grim CE, Miller JZ, Luft FC, Christian JC, Weinberger MH (1979): Genetic influences on renin, aldosterone, and the renal excretion of sodium and potassium following volume expansion and contraction in normal man. Hypertension 1:583–590.

Jinks JL, Fulker DW (1970): Comparison of the biometrical genetical, MAVA and classical approaches to the analysis of human behavior. Psychol Bull 73:311–349.

Kendler KS (1983): Overview: A current perspective on twin studies of schizophrenia. Am J Psychiatry 140:1413–1425.

Liu K, Cooper R, McKeever J, McKeever P, Byington R, Soltero I, Stamler R, Gosch F, Stevens E, Stamler J (1979): Assessment of the association between habitual salt intake and high blood pressure: Methodological problems. Am J Epidemiol 110:219–226.

MacGregor GA, Markandu ND, Best FE, Elder DM, Cam JM, Sagnella GA, Squires M (1982a): Double-blind randomised cross-over trial of moderate sodium restriction in essential hypertension. Lancet i:351–355.

MacGregor GA, Smith SJ, Markandu ND, Banks RA, Sagnella GA (1982b) Moderate potassium supplementation in essential hypertension. Lancet i:567–570.

Martin NG (1975): The inheritance of scholastic abilities in a sample of twins: II. Genetical analysis of examination results. Ann Hum Genet 39:219–229.

Martin NG, Eaves LJ, Kearsey MJ, Davies P (1978): The power of the classical twin study. Heredity 40:97–116.

Morgan T, Myers J, Carney S (1979): The evidence that salt is an important aetiological agent, if not the cause, of hypertension. Clin Sci 57:459s–462s.

Shephard MDS, Penberthy LA, Fraser CG (1981): Short- and long-term biological variation in analytes in urine of apparently healthy individuals. Clin Chem 27:569–573.

Simpson FO (1979): Salt and hypertension: A sceptical review of the evidence. Clin Sci 57:463s–480s.

Tobian L (1979): The relationship of salt to hypertension. Am J Clin Nutr 32:2739–2748.

Weisinger RS, Conside P, Denton DA, McKinley MJ, Mouw D (1979): Rapid effect of change in cerebrospinal fluid sodium concentration on salt appetite. Nature 280:490–491.

Edited by D.C. Rao