RELATIVE ROLES OF GENETIC AND ENVIRONMENTAL FACTORS IN THE CONTROL OF SERUM URIC ACID LEVELS IN NORMOURICAEMIC SUBJECTS

BY

JAMES A. BOYLE, WILLIAM R. GREIG, MUKUNDRAI K. JASANI, ANNE DUNCAN,
MICHAEL DIVER, AND W. WATSON BUCHANAN

From the Centre for Rheumatic Diseases, Glasgow, the University Department of Medicine, and the Department of Biochemistry, Royal Infirmary, Glasgow, Scotland

Gout has long been thought to be hereditary and several genetic hypotheses have been proposed for the occurrence of hyperuricaemia (Smyth, Cotterman, and Freyberg, 1948; Stecher, Hersh, and Solomon, 1949; Hauge and Harvald, 1955). Blumberg (1965), in a critical review of these studies, has concluded that they are far from ideal in testing a genetic hypothesis, and comments that this may be why such studies have provided inconsistent results. Indeed, recently, O'Brien, Burch, and Bunim (1966), in a carefully controlled epidemiological study of the Blackfeet Indians in Montana and the Pima Indians in Arizona, showed that the trait of hyperuricaemia "is for the most part not a genetic one but is instead largely determined by environmental factors". These authors, did, however, note some familial aggregation of hyperuricaemia, but genetic analysis was not consistent with either a simple recessive or dominant inheritance; analysis of a polygenic trait indicated a rather low heritability.

It occurred to us that it might be of interest, in view of the present controversy surrounding the genetics of gout and hyperuricaemia, to ascertain the strength of the genetic and environmental components in the control of the serum uric level in normouricaemic subjects.

To do this we have adopted the twin study method where the mean intrapair variance of serum uric acid levels observed in monozygotic (MZ) (genetically-identical) twin pairs has been contrasted with the mean intrapair variance of serum uric acid levels in dizygotic (DZ) (genetically non-identical) twin pairs and the results of this comparison have been analysed by calculating the variance ratio and assessing its significance on the F ratio, as has been described by Osborne and De George (1959).

Material and Methods

112 twin pairs were studied. The methods of ascertainment and determination of zygosity have been described

in detail elsewhere (Buchanan, Boyle, Greig, McAndrew, Barr, Anderson, and Goudie, 1966; Greig, Boyle, Duncan, Nicol, Gray, Buchanan, and McGirr, 1967). The likelihood of a correct diagnosis of monozygosity is greater than 90 per cent. for every twin pair accepted as being MZ in the present study (Dencker, Hauge, Kaij, and Nielsen, 1961), and in the case of the DZ twins the likelihood of a correct diagnosis is 100 per cent. because dizygosity was diagnosed only when there was unequivocal evidence, such as different iris colour or blood group antigen. The ascertainment campaign which was used to obtain twins mentioned rheumatoid arthritis, but did not mention gout, and no twins in this study had gout.

Each twin pair were seen together and blood samples were taken at the same time in the non-fasting state. None of the subjects had ingested alcohol on the day of the study but we have no information on the drugs, such as salicylates, which this essentially healthy population of twins might have been taking. As the numbers of twin pairs are fairly large we feel that, although it is possible that a few may have been taking salicylates or other drugs known to alter blood uric acid levels, it is unlikely that this factor would be of much importance in influencing the conclusions of the study. None of the twins had clinically apparent disease, all had normal blood pressures and all but eleven individuals had a serum creatinine level < 1.00 mg. per cent. These eleven individuals have been included in the study since none had evidence of renal disease; and these subjects presumably are reasonably representative of the normal population.

The numbers of twin pairs studied in this investigation are shown in Table I (overleaf). The breakdown of the 112 twin pairs studied according to sex and zygosity shows an increase in the number of MZ female twin pairs, which has been the experience of other workers who have obtained twins by an ascertainment campaign (Osborne and De George, 1959). It can also be seen that the mean age and age ranges were similar in all groups. As no twins under the age of 12 years were studied it will be appreciated from the mean and standard deviation of the ages that the majority were young; indeed, 71 twin pairs were under the age of 20 years.

The serum uric levels were determined by an automated colorimetric method using the Technicon Auto Analyser

TABLE I
RESULTS OF SERUM URIC ACID DETERMINATIONS IN 112 TWIN PAIRS

Twin Group				No. of Twin Pairs	Age (yrs)			Results of Serum Uric Acid Determinations (mg./100 ml.)			
					Mean	S.D.†	Range	Mean	S.D.†	Range	Intrapair* Variance
Monozygotic	Female	..	..	35	21	12.6	12-66	3.65	0.87	1.30-5.30	0.173
	Male	..	..	16	25	17.6	12-65	4.87	1.01	3.00-7.20	0.402
Dizygotic	Female	..	..	20	25	16.3	12-70	3.66	1.05	0.50-5.70	0.898
	Male	..	..	11	23	13.2	13-47	4.59	1.43	2.20-7.20	0.160
Dizygotic Unlike-Sex				30	25	15.5	13-64	4.03	0.85	2.10-6.00	0.49

†S.D. = Standard Deviation

*See text for explanation of how this function was calculated.

Method No. 22. All sera were stored at -20°C . for 3 weeks before estimation of the serum uric acid, because Buchanan, Isdale, and Rose (1965) have shown that chromogens present in fresh sera which give a falsely high reading in colorimetric methods measuring uric acid largely disappear from the sera after storage for 3 weeks in the frozen state.

The calculation of interpair variances for the twins was performed as described by Osborne and De George (1959). Essentially the method contrasts the variance of the sums of differences between MZ twins with the variance observed for these functions in DZ twins. The significance of the resulting figure is tested on the F ratio distribution. Full details of this method are described by Osborne and De George (1959) and Mathers, Osborne, and De George (1961). Allen (1966), in a review of the problems in twin research, has commented that when one is dealing with the continuous variables the variance ratio method is preferable to the heritability index which we have used for dealing with discrete variables in previous studies (Buchanan and others, 1966; Greig and others, 1966).

Results

The results are summarized in Tables I and II.

The mean serum uric acid levels were 3.65 and 3.66 mg./100 ml. for the MZ and DZ female twin pairs respectively, and 4.87 and 4.59 mg./100 ml. for the MZ and DZ male twin pairs respectively.

The range of serum uric acid levels in female twins

TABLE II
SIGNIFICANCE VALUES FOR OBSERVED INTRAPAIR VARIANCES IN MALE AND FEMALE TWINS

Twin Group		Intrapair Variance*	F ratio*	P value
Female	Monozygotic	0.173 } 0.898 }	5.19	<0.001
	Dizygotic			
Male	Monozygotic	0.402 } 0.160 }	0.40	Not significant
	Dizygotic			

* See text for explanation of how this function was calculated.

was 0.50-5.7 mg./100 ml. and in male twins 2.2-7.2 mg./100 ml. The singularly low value of 0.5 mg./100 ml. was present in a 19-year-old female DZ twin. It has been shown that low serum uric acid levels are present as early as 12 weeks in normal pregnancy (Boyle, Campbell, Duncan, Greig, and Buchanan, 1966), and it may be that this twin was pregnant but we have no information on this point.

The intrapair variance of 0.173 in the MZ female twin pairs contrasts with the intrapair variance of 0.898 in the DZ female twin pairs. This comparison yields an F ratio of 5.19 which is significant at the less than one in a thousand level (Table II).

The intrapair variance for the MZ male twin pairs (0.402) was higher than the intrapair variance for the DZ male twin pairs (0.160), which is clearly not significant of a genetic component (Table II).

Table III (overleaf) contrasts the intrapair variance of serum uric acid levels in all twins, in twins living together in the same household, and twins living apart from each other for longer than one year. The mean intrapair variance in MZ female twins was 0.08 in those living together and 0.70 in those living apart. This difference is highly significant ($P < 0.01$) and is indicative of an environmental factor in control of serum uric acid level in females. For the DZ female twins these figures were 0.41 (living together) and 1.58 (living apart) which is further support of this conclusion. In MZ male twin pairs the variance was slightly higher in those living apart (0.56) than in those living together (0.33). The number of DZ male twin pairs living apart, however, was very small. Interpretation of the results in the unlike-sex DZ twin pairs is difficult because of the sex difference: the mean intrapair variance in those living apart was lower (0.50) than those living together (0.82), but these differences are not statistically significant.

TABLE III
MEAN INTRAPAIR VARIANCE* OF SERUM URIC ACID LEVELS IN TWINS LIVING TOGETHER AND IN TWINS LIVING APART (SEPARATED FOR 1 YEAR OR LONGER)

Twin Group		Twins Living Together					Twins Living Apart				
		No. of Twin Pairs	Age (yrs)			Intrapair Variance* of Serum Uric Acid Levels	No. of Twin Pairs	Age (yrs)			Intrapair Variance* of Serum Uric Acid Levels
			Mean	Range	S.D.†			Mean	Range	S.D.†	
Female	Monozygotic	29	18	12-50	7.4	0.08	6	37	19-66	20.4	0.70
	Dizygotic	14	19	12-70	15.2	0.41	6	38	27-50	9.4	1.58
Male	Monozygotic	11	15	12-21	2.7	0.33	5	48	30-65	15.6	0.56
	Dizygotic	8	15	13-17	1.7	0.16	3	43	37-47	4.9	0.15
Dizygotic Unlike-Sex		19	18	13-55	9.2	0.82	11	39	23-64	4.7	0.50

†S.D. = Standard Deviation

*See text for explanation of how this function was calculated

Discussion

To our knowledge only one previous study of serum uric acid levels in normal twins has been reported (Jensen, Blankenhorn, Chin, Sturgeon, and Ware, 1965). These authors studied thirteen MZ males, fourteen MZ females, six DZ males, seven DZ females, and four unlike-sex DZ twins, and interpreted their findings as providing "more conclusive evidence for genetic control of . . . uric acid in normal man that has been available previously". Interpretation of their data, however, is difficult for the following reasons. The numbers studied were relatively small. They included four unlike-sex DZ twins when comparing the intrapair variance of MZ to DZ twins. Such a procedure is unwarranted because the sex differences known to exist in serum uric acid levels make it likely that the intrapair variance of DZ unlike-sex twins will be higher than the intrapair variance of either MZ or DZ like-sex pairs, as has been discussed by Osborne and De George (1959).

A factor which should cause hesitation before one too readily ascribes a significantly smaller intrapair variance in MZ compared with DZ twin pairs to the effects of genetic control of these variances, is the possibility that MZ twins may share a closer common environment than do DZ twins. The lower MZ intrapair variance might then merely be a reflection of this closeness of environment. It is unlikely that such considerations apply in the present study, for the significantly lower intrapair variance for serum uric acid values in MZ females compared with DZ females, was not paralleled by the findings in the MZ and DZ male twins and the vast majority of twins of both sexes were living together at the time of the study. The composition of the 31 twin pairs living apart was as follows: six female MZ, six

female DZ, five male MZ, three male DZ, and eleven unlike-sex DZ pairs.

It could be argued that the fact that males have a higher serum uric acid level than females (Seegmiller, Laster, and Howell, 1963) might cause the intrapair difference to be greater in male twins than in female twins. However, as one is concerned with the *ratio* of the variances of the intrapair differences in MZ and DZ twins within each sex, this objection does not explain the strikingly high F value found in the female twins compared with male twins.

It seems reasonable to conclude, therefore, that the smaller intrapair variance in the female MZ compared to DZ twins almost certainly reflects a fairly strong genetic control of the variation in serum uric acid levels in females. The failure to demonstrate a genetic control of the normal serum uric acid level in the male does not mean that such control does not exist but merely that it is likely to be weak and that there is a very strong environmental factor (or factors) operating in males in control of the serum uric acid level.

Many environmental factors are now recognized in the control of serum uric acid levels and these have been discussed by Seegmiller, Laster, and Howell (1963). The results of the present study suggest that the mechanisms controlling the normal serum uric acid levels are more influenced by an environmental factor (or factors) in males, and that females are for some reason (either environmental or genetic) less susceptible to this factor. That variations in the level of serum uric acid in females are also influenced by environmental factors is clear from the study of intrapair variances in female twins living together and living apart (Table III).

The nature of this environmental factor (or factors) which predominates over genetic factors in males but not in females in controlling serum uric acid

levels is not yet known, for no one has so far to our knowledge described a differing reaction between the sexes to an environmental influence on serum uric acid levels.

It is known that males have a higher uric acid level than females and that primary hyperuricaemia is commoner in men than in women. It is tempting to speculate that this situation may be partly explained by the reaction of males to environmental factors which women either do not meet or to which they are genetically less susceptible. The present study neither confirms nor refutes this speculation and it would clearly be of value in further work to document all cases of MZ males discordant for hyperuricaemia. If these were found in any numbers and the reason for the hyperuricaemia fully documented (whether overproducers or underexcretors of uric acid) our knowledge of the genetics of hyperuricaemia would be much enlarged.

Summary

Serum uric acid levels were estimated by an automated colorimetric method in 112 pairs of healthy twins from the Glasgow and West of Scotland area. Calculation of the intrapair variances in monozygotic and dizygotic twins showed a significant

genetic component in the control of variation in the serum uric acid level in females. The study failed to detect a strong genetic component in males. The possibility that an environmental factor (or factors) plays an important role in the maintenance of the higher serum uric acid level found in males compared with females is discussed.

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Rôles relatifs des facteurs génétiques et du milieu ambiant dans le maintien du niveau de l'acide urique sanguin chez des sujets normouricémiques

RÉSUMÉ

On détermina le taux sérique de l'acide urique par une méthode colorimétrique automatique chez 112 paires de jumeaux sains de la ville Glasgow et de l'Ecosse de l'Ouest. Le calcul des variances *intrapaires* pour les jumeaux monozygotes et dizygotes démontra l'existence d'un composant génétique significatif affectant le taux de l'acide urique sanguin chez les femmes. On ne réussit à déceler aucun composant génétique fort chez les hommes. On discute la possible existence d'un facteur (ou facteurs) du milieu ambiant jouant un rôle important dans le maintien des taux uricémiques plus élevés chez les hommes par rapport aux femmes.

Papeles relativos de los factores genéticos y del medio ambiente en el mantenimiento del nivel del ácido úrico en el suero de sujetos normouricémicos

SUMARIO

Se determinó por un método colorimétrico automatizado el nivel del ácido úrico en el suero de 112 parejas de gemelos sanos de la ciudad de Glasgow y de Escocia occidental. La calculación de las variaciones *intra-gemelas* en parejas mono y dicigóticas reveló la existencia de un componente genético significativo afectando las cifras del ácido úrico en el suero de las mujeres. No se llegó a descubrir tal componente en el suero de los hombres. Se discute la posible existencia de un factor (o factores) del medio ambiente desempeñando un papel importante en el mantenimiento del nivel uricémico más alto en los hombres que en las mujeres.