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ABH SALIVARY SECRETION IN TWINS

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SUMMARY

Fifty MZ and 51 same-sex DZ twins were studied for ABH salivary secretions and the amount of these substances measured in those with the same A_1A_2BO blood groups. Using the classical, manual inhibition test we verified: (*a*) Levels for the H antigen in A_2 and B persons similar to, but in O and A_1 lower than those obtained in other places; (*b*) Low averages for the A and B substances; (*c*) Mean intrapair differences for A and H, which were the double in DZ as in MZ twins, the estimated degree of genetic determination being 0.86 and 0.69 respectively; in both cases the *F*-ratio was significant at the 0.01 level; (*d*) If one discrepant family is disregarded the correlation coefficient between O parents and DZ children for the H antigen (0.48) is of the order of magnitude expected under a simple additive model of polygenic inheritance.

Tests were also made using a fully automated method. The intraclass correlation coefficients thus obtained in MZ and DZ were similar to those reached using the data obtained with the classical technique. The degree of concordance arrived at by the two methods was only fair, but this may have been due to extraneous factors related to the way the samples were handled.

Despite the fact that it is now known for forty years that the presence of ABH substances in the saliva is due to one pair of genes (Schiff and Sasaki 1932), not much information is at hand about the factors that influence the level of these antigens in this fluid. Family studies were performed by Clarke et al. (1960) and Plato and Gershowitz (1962) while twin investigations were done by Matsuzawa et al. (personal communication to Matsunaga and Susuki 1958), and Surneva (1970). These researches indicated that the amount of these substances present in a given individual is also under genetic control; but the details about how this is accomplished are not well defined, and the number of subjects studied is small.

The present investigation was undertaken to furnish additional data about this problem and is related to a more general project aiming at establishing the relative roles of heredity and environment in a series of morphological, physiological and psychological characteristics. A first paper about some of these results has already been published (da Rocha et al. 1972). With the development of a fully automated continuous-flow hemagglutination inhibition machine, which permits a more efficient quantitative estimate of antigen levels (Sturgeon and Mc Quiston 1967), an opportunity arose to compare the data we had obtained using the traditional manual methods with those more rigorous determinations.

MATERIALS AND METHODS

A total of 50 MZ and 51 same-sex DZ twins were ascertained primarily through inquiries at high-schools; but indications obtained from the twins themselves about other pairs, and other sources of information were also employed for the localization of the series. With the exception of two male DZ pairs who came from the nearby cities of Esteio and Canoas, all the others were living in Porto Alegre, RS, Brazil. Twenty-one percent of the twins proved to be nonsecretors, in good agreement with a population sample obtained previously in the latter city (Palatnik et al. 1969). The twins' A_1A_2BO frequencies also showed values similar to those obtained previously in independent samples from this population (Salzano 1963, Salzano et al. 1967). A subsample of 38 MZ and 26 DZ pairs who were secretors and concordant in relation to the above-mentioned blood group system, was then selected for further study. Their mean age was 20 years; sex distribution: MZ, 19 male and 19 female; DZ, 11 male and 15 female; 2 DZ male pairs were classified as Mulattoes, all the others being White.

The twins' zygosity was determined using blood groups A_1A_2BO, Rh (tests with anti-C, $-C^w$, -c, -D, -E, -e), MNSs, P, Duffy and Kell; ABH secretion; and serum haptoglobins. Tests for color-blindness were also performed but none of the twins proved to be deficient. Standardized photographs and dermatoglyphics were obtained as well, but were not considered in the final decision about zygosity. A pair was classified as dizygotic if it presented any difference in the above mentioned genetic markers. The probability of dizygosis in twins concordant for all of them varied from 2 to 6 per cent. However, it should be noted that only in relation to one pair there was doubt about the classification made, and in this case the inclusion of dermatoglyphic traits in the probability estimates strengthened the classification based on monogenic characteristics.

To obtain further information about the genotypes of the DZ pairs in relation to the *Se/se* polymorphism, 39 parents of 22 pairs were also tested for the ABO blood groups and ABH secretion.

The salivas were collected in paper cups, transferred to tubes, boiled for 20 minutes and centrifuged. They were then stored at -10° C until tested. The presence of ABH substances was determined by the inhibition of commercial human anti-A and -B (Johnson and Johnson of Brazil) and of the anti-H of *Ulex europaeus*, the latter prepared according to the method described by Boyd and Shapleigh (1954). They were used in a concentration which was the double of the titer that gave the maximum agglutination with the fresh A_2, B, or O red cells subsequently employed in the tests.

The quantitative determinations were carried out by using double dilutions of saliva in saline from neat to 1 in 16,384 (15 tubes). An equal volume of antiserum was added to each tube and the mixture kept at room temperature for half an hour. Appropriate red cells were then added and the readings done macroscopically after centrifugation. The inhibition titer was recorded as the last dilution in which there was complete neutralization. A control saliva was always tested in parallel and its titers never varied more than one dilution in determinations performed at different times. All readings were made without knowing the zygosity of the twins who were being tested; the salivas of members of each pair were always studied simultaneously.

Determinations were also carried out using a recently developed fully automated continuous-flow hemagglutination inhibition system for the quantitation of A, B, and H substances (Sturgeon and Mc Quiston 1967). The salivas were sent under refrigeration to Los

Angeles where Dr. Sturgeon had kindly agreed to perform the automated tests. Unfortunately, the conditions during the transportation were not very satisfactory so that no more ice existed at their arrival. Nevertheless they were immediately placed in the freezer until tested. Due to an oversight they remained there for some months before they were finally analysed.

RESULTS

Tables I and II compare the levels of H, A, and B antigens of our sample, as determined by manual techniques, with those from other populations. In relation to H the averages in A_2 and B persons are similar to the ones observed in Ann Arbor; but those encountered in O and A_1 individuals are lower than those obtained in other places. Low values were also found in relation to the A and B substances.

Estimates of the degree of genetic determination of the A and H levels using the manual and automated methods are shown in Tables III and IV, respectively. The analysis of the intrapair differences in MZ and DZ twins involved, of course, only

TABLE I

H ANTIGEN IN THE SALIVA OF PORTO ALEGRE TWINS AND OTHER POPULATION SAMPLES

Blood group	No. studied, mean, and standard deviation	Transformed (-1 x $\log_2$) H inhibition titer			
		Porto Alegre[a]	Japan[b]	Ann Arbor USA[c]	Aligarh India[d]
O	N	43	343	50	40
	$\bar{x}$	5.8	7.9	8.0	8.7
	s^2	1.7	4.8	2.2	2.1
A_2	N	10	—	50	—
	$\bar{x}$	6.3	—	6.3	—
	s^2	1.2	—	1.9	—
A_1	N	30	—	50	—
	$\bar{x}$	4.1	—	5.2	—
	s^2	1.4	—	1.8	—
B	N	14	—	50	—
	$\bar{x}$	3.1	—	3.9	—
	s^2	1.1	—	1.6	—
AB[e]	N	3	—	50	—
	$\bar{x}$	2.0	—	3.8	—
	s^2	—	—	1.6	—

[a] For these calculations we have chosen at random only one member of each pair. Tests with anti-H of *Ulex europaeus*.

[b] Matsunaga and Susuki (1958). Chicken anti-H.

[c] Plato and Gershowitz (1961). Anti-H of *Ulex europaeus*.

[d] Tyagi et al. (1968). Anti-H of *Ulex europaeus*.

[e] Two A_1B and one A_2B in the sample from Porto Alegre.

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TABLE II

A AND B ANTIGEN IN THE SALIVA OF PORTO ALEGRE TWINS AND OTHER POPULATION SAMPLES

Blood group	No. studied, mean, and standard deviation	Transformed (-1 x $\log_2$) inhibition titer				
		Porto Alegre[a]		New York, USA[b]	Aligarh, India[c]	
		A	B	A	A	B
A_1	N	30	—	17	92	—
	$\bar{x}$	8.2	—	11.4	10.9	—
	s^2	1.5	—	1.4	2.4	—
A_2	N	10	—	4	—	—
	$\bar{x}$	6.3	—	12.0	—	—
	s^2	2.1	—	—	—	—
AB[d]	N	3	3	3	29	33
	$\bar{x}$	6.0	7.7	9.0	10.5	11.4
	s^2	—	—	—	3.5	2.4
B	N	—	14	—	—	132
	$\bar{x}$	—	8.8	—	—	11.6
	s^2	—	1.1	—	—	2.4

[a] For these calculations we have chosen at random only one member of each pair.

[b] Wiener and Kosofsky (1941).

[c] Tyagi et al. (1968). No distinction was made in this sample between A_1 and A_2 persons.

[d] Two A_1B and one A_2B in the sample from Porto Alegre.

individuals who had the same blood group and subgroup. Since in only one DZ pair both members were secretors and concordant for B, no such estimates could be made for this antigen. In order to increase the sample size, data from the A_1, A_2, A_1B, and A_2B twins were pooled for the study of the A substance; a similar procedure was employed in relation to the H antigen, using the results of all groups.

As is shown in Table III, with the manual method the mean intrapair differences for both substances proved to be the double in DZ as in MZ twins. The correlation coefficients were accordingly higher and the within-pair variances lower among the latter, leading to h^2 estimates of 0.86 for A and 0.69 for H. In both cases the F-ratio was significant at the 0.01 level. Similar analyses were made considering the groups and subgroups separately. When the number of individuals allowed comparisons, the r, h^2 and F values were not very different from those of the pooled sample.

Somewhat fewer pairs were tested with the automated technique (Table IV). The correlation coefficients were also higher among MZ but in this case the intrapair variance for A showed an anomalously large value, preventing a reliable h^2 estimate; as for the H substance, the results are more in accordance with the expectation, but the F-ratio is not significant. This discrepancy is reflected in the correlation coeffi-

TABLE III

GENETIC DETERMINATION OF A AND H ANTIGENS IN THE SALIVA OF PORTO ALEGRE TWINS
(*Manual Method*)

Antigen	Zygosity	No. of pairs	Mean intrapair difference	Variance		r	h^2	F
				Between	Within			
A	MZ	17	0.4	3.09	0.21	0.88***	0.86	6.98**
	DZ	8	1.1	6.56	1.44	0.64		
H	MZ	38	0.4	7.38	0.21	0.94**	0.69	3.20**
	DZ	26	0.8	6.72	0.67	0.82***		

** Significant at the 0.01 level.
*** Significant at the 0.001 level.

TABLE IV

GENETIC DETERMINATION OF A AND H ANTIGEN IN THE SALIVA OF PORTO ALEGRE TWINS
(*Automated Technique*)

Antigen	Zygosity	No. of pairs	Mean intrapair difference	Variance		r	h^2	F
				Between	Within			
A	MZ	14	29.4	9685.95	1015.08	0.81***	0.00	0.51
	DZ	8	28.8	1907.60	519.95	0.57		
H	MZ	30	8.0	540.26	71.87	0.77***	0.21	1.27
	DZ	24	9.7	406.76	91.21	0.63***		

*** Significant at the 0.001 level.

cients obtained comparing the results of the two methods. They are as follows: antigen A: MZ 0.12, DZ 0.58; H: MZ 0.20, DZ 0.27. Total: 0.22. Despite the fair degree of correlation, the two sets of data do not agree very well.

Matsunaga (1959) suggested that the homozygotes *Se/Se* present higher levels of ABH substances in the saliva than *Se/se* individuals. The influence of this locus can be indirectly ascertained by studying the parents of DZ twins. Therefore an effort was made to test all those 52 parents; unfortunately it was not possible to study 13 of them (25%). These investigations gave indications of genotypic concordance in 3 of the 19 pairs with both parents tested (16%); theoretically we would expect a clear indication in 35% of the cases.

The information obtained from the DZ twins' parents furnished additional data about the genetic determination of the level of the H substance in saliva. In 38 comparisons between group-O parents and children the observed correlation coefficient was 0.25. This low value, however, was due to a large extent to a single family, in which the parents' titers were much lower than those of the twins. When these four persons were withdrawn from the sample, the obtained correlation was much higher: 0.48.

DISCUSSION

The low levels of H, A, and B substances observed in our sample, as compared to those found in US, Japanese, and East Indian groups, may be due to different frequencies of the genes inolved in their production; to the fact that we tested twins; or to methodological problems, such as the use of antibodies of different strengths or interobserver variations. Studies in twin series from other populations may provide data which would allow a decision among these alternatives.

Our results with the manual technique indicate a relatively high influence of the genotype in the regulation of the levels of A and H substances in saliva; the estimates of the degree of genetic determination obtained for these two antigens were respectively 0.86 and 0.69, while the sib-parent correlation coefficient in relation to H was close to 0.50. These values are in accordance with those obtained by other authors. Thus, Clarke et al. (1960) and Plato and Gershowitz (1962) found correlation coefficients between sibs ranging from 0.35-0.66, while Surneva (1970) observed r values close to 1.00 in MZ and from 0.18-0.85 in DZ. Unfortunately, the twin method cannot provide an answer to the question whether this genetic influence is due to multiple allelism or independent modifiers. New family data are necessary to discriminate between these two hypotheses.

The figures obtained with the automated method showed only a fair degree of concordance with those arrived at with the manual one. This may be due to many factors, the most important probably being transportation problems and prolonged storage; the latter may have led to a partial denaturation of these substances and to increased within-pair variances.

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Riassunto

Cinquanta coppie di gemelli MZ e 51 DZ dello stesso sesso sono state studiate rispetto alla secrezione salivare di sostanze ABH: la quantità di tali sostanze è stata studiata in quelle coppie che presentavano gli stessi gruppi sanguigni A_1A_2BO. Mediante il classico test dell'inibizione manuale sono stati verificati: (*a*) i livelli dell'antigene H simili a quelli ottenuti altrove per gli individui A_2 e B, ma inferiori per gli individui O e A_1; (*b*) medie basse per le sostanze A e B; (*c*) differenze medie intracoppia pari al doppio nei gemelli DZ rispetto ai MZ, con livello di determinazione genetica rispettivamente di 0.86 e 0.69; in ambedue i casi il rapporto *F* era significativo al livello dello 0.01; (*d*) se si esclude una famiglia che fa eccezione, il coefficiente di correlazione fra genitori O e figli gemelli DZ per l'antigene H (0.48) è dell'ordine di grandezza atteso nell'ipotesi di un modello additivo semplice di eredità poligenica.

I test sono anche stati eseguiti con un metodo completamente automatizzato. I coefficienti di correlazione intraclasse in tal modo ottenuti in gemelli MZ e DZ sono risultati simili a quelli ottenuti con il test classico. Il grado di concordanza raggiunto dai due metodi è limitato, ma ciò può essere dovuto a fattori estranei legati al modo in cui i campioni sono stati trattati.

Résumé

Cinquante couples de jumeaux MZ et 51 DZ du même sexe ont été étudiés pour la sécrétion salivaire de ABH: la quantité de ces substances a été étudiée chez les couples ayant les mêmes groupes sanguins A_1A_2BO.

Moyennant le test classique de l'inhibition manuelle ont été vérifiés: (*a*) des niveaux de l'antigène H, similaires qu'ailleurs pour les individus A_2 et B mais inférieurs pour les sujets O et A_1; (*b*) de basses moyennes pour les substances A et B; (*c*) des différences intracouple moyennes doubles chez les DZ vis-à-vis des MZ, avec un niveau de détermination génétique respectivement de 0.86 et 0.69; dans les deux cas le rapport *F* était significatif au niveau de 0.01; (*d*) si l'on exclut une famille qui fait exception, le coefficient de corrélation entre parents O et fils jumeaux DZ pour l'antigène H (0.48) est dans l'ordre de grandeur attendu dans l'hypothèse d'un modèle additif simple d'hérédité polygénique.

Les tests ont aussi été exécutés par une méthode complètement automatisée. Les coefficients de corrélation intraclasse ainsi obtenus chez les jumeaux MZ et DZ ne diffèrent pas de ceux obtenus par la méthode classique. Le degré de concordance des deux méthodes est limité, mais ceci peut être dû à d'autres facteurs liés à la façon dont les échantillons ont été traités.

ZUSAMMENFASSUNG

Fünfzig EZ und 51 gleichgeschlechtliche ZZ-Paare wurden auf den ABH-Gehalt im Speichel untersucht, und bei den Paaren mit Konkordanz der Blutgruppen A_1A_2BO wurde die Mengenbestimmung dieser Stoffe vorgenommen. Mit Hilfe des klassischen handbetriebenen Hemmungstests wurden folgende Ergebnisse erzielt: (*a*) Die H-Antigenniveaus entsprachen denen, die anderweitig für Träger der Blutgruppen A_2 und B beobachtet wurden, waren aber niedriger als bei den Blutgruppen O und A_1; (*b*) niedrige Durchschnittswerte der Stoffe A und *B*: (*c*) die durchschnittlichen Unterschiede zwischen den beiden Paarlingen waren bei ZZ doppelt so hoch wie bei EZ, mit einer Erbbedingtheit von 0.86 bzw. 0.69; bei beiden Fällen war das Verhältnis *F* auf Niveau 0.01 bedeutungsvoll; (*d*) mit Ausnahme einer Familie entspricht der Korrelationskoeffizient zwischen O-Eltern und ZZ-Kindern für das H-Antigen (0.48) der Erwartung für ein einfaches additives, polygenes Erbmodell.

Die Tests wurden auch mit einer völlig automatisch betriebenen Methode durchgeführt. Die auf diese Weise erhaltenen Korrelationskoeffizienten für die Paarlingsunterschiede bei EZ und ZZ entsprechen denen des klassischen Tests. Der mit beiden Methoden erzielte Konkordanzgrad ist beschränkt, was jedoch durch äussere mit der Behandlung der Muster zusammenhängende Faktoren bedingt sein könnte.

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