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ABO SALIVA AND PLASMA AGGLUTININS IN TWINS

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SUMMARY

The levels of ABO plasma agglutinins were studied in 47 MZ and 30 same-sex DZ pairs of twins having the same blood group. The degree of genetic determination of this characteristic, as ascertained by the comparison of average titers and titration scores, does not seem to be high; despite the fact that the correlation coefficients were always lower among DZ, the *F* ratio was significant in relation to the anti-B of A persons only. The presence of these antibodies was also investigated in the saliva of 30 MZ and 26 DZ pairs. Since about the same degree of discordance was observed among these two types of twins, the role of inheritance in the variability of this trait must be low.

There is much discussion about the factors that influence the levels of ABO plasma and saliva agglutinins in a given individual. One of the main questions that have to be answered is whether the secretory immunoglobulin system is or is not distinct from the system producing circulating antibodies (Prokop 1961*b*, Claman et al. 1967, Bell and Fortwengler 1971). Wide variations may also be detected in the salivary isoagglutinin titers of a person at different times (see, for instance, Nebert 1962, Otten 1966). Due to these and other problems, studies about the genetic determination of these saliva agglutinins are not numerous and have led to contradictory results (Miyakoshi 1951, Furuhata et al. 1959, Schmitz-von Hülst and Clausnitzer 1960, Wilson and Green 1964, Boettcher 1967); we have not been able to locate any previous study dealing with twins, either. Investigations about the genetic factors which would influence the levels of ABO plasma agglutinins are also few, showing discordant date (Bühler 1935, Dahr 1941, Furuhata and Matsunaga 1952, Nijenhuis and Bratlie 1962). Therefore we decided to include these traits in a general investigation about morphological, physiological and psychological characteristics in twins (for previous analyses, see De Rocha et al. 1972, Callegari et al. 1972).

MATERIALS AND METHODS

Fifty MZ and 51 same-sex DZ pairs of twins raised together were ascertained mainly at high-schools, other sources of information also being employed. 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. The twins' A_1A_2BO frequencies showed values similar to those obtained previously in independent samples from this population (Salzano 1963,

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Salzano et al. 1967). Their zygosity was determined by 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. A pair was classified as DZ if it presented any difference in the above-mentioned genetic markers. The probability of dizygosis in the twins concordant for all of them varied from 2 to 6 per cent. However, only in relation to one pair there was doubt about its zygosity, and the analysis of the twins' dermatoglyphics strengthened the first classification.

The salivas were collected in paper cups, transferred to tubes and frozen immediately at 10° C until tested. The determinations were done after centrifugation, in undiluted saliva placed in 75 × 10 mm tubes to which an equal volume of 2 per cent A_1 or B red cells in saline were added. Readings were performed macroscopically after one hour at room temperature, without centrifugation. These studies were started only after the investigation of 23 pairs had been finished; therefore fewer individuals were included. In addition, problems of storage and of the saliva's isotonicity prevented the proper study of many twins. The final subsample for the saliva agglutinin results was, therefore, only 30 MZ and 26 DZ pairs. Their mean age was 19 years; sex distribution: MZ, 12 male and 18 female; DZ, 10 male and 16 female; two pairs (one MZ, and one DZ) were Mulattoes, all the others being White.

The collection of blood was made in 10 ml vacutainers with EDTA. The plasmas were separated immediately or one or two days afterwards and kept at -10° C until tested. Their study involved double dilutions in saline from neat to 1 in 16384 (15 tubes). A_1 or B cells were then added, the mixture centrifuged, and the readings done macroscopically. The titer was recorded as the $\log_2$ of the reciprocal of the greatest dilution of the serum to give a positive reaction. A titration score was also calculated as described by Dunsford and Bowley (1967), 10 different degrees of agglutination being distinguished. For these studies 47 MZ and 30 DZ pairs having the same A_1A_2 BO blood group were selected. Their mean age was 20 and 19 years respectively for the MZ and DZ; sex distribution: MZ, 20 male and 27 female; DZ, 12 male and 18 female. Three pairs (one MZ and two DZ) were Mulattoes, all the others being White.

Both for the saliva and plasma determinations the readings were made without knowing the zygosity of the twins who were being tested; the samples of members of each pair were always studied simultaneously.

RESULTS

The results obtained in Porto Alegre for the presence of anti-A and anti-B in the saliva of 56 pairs of twins are compared with data from other authors in Table I. In our sample individuals with blood group O presented agglutinins in a higher (68%) frequency than B (43%) or A (24%) persons. Considering all these individuals simultaneously we obtain a prevalence of 48%. Very different methods were utilized in the other series, and this could explain, at least in part, the wide variability observed. The most extreme figures were those of Hummel and Schöch (1967) who found agglutinins in 100% of their subjects by using a method of multiple incubations. However, in the first of their multiple incubations their results fall within the range of variation observed by the remaining investigators, which was 5-59% in group A, 12-62% in group B and 31-90% in group O. It should also be noted that in 6 of the 8 series which

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Table I

Presence of Anti-A and Anti-B in the Saliva of A, B and O Individuals from Different Series

Reference		Method [a]	A		B		O		Total	
			No. studied	% with anti-B	No. studied	% with anti-A	No. studied	% with anti-A and/or anti-B	No. studied	% with ag-glutinins
Present communication [b]		I	21	24	7	43	28	68	56	48
Schmitz-von Hülst and Clausnitzer	1960	II	n.g.	n.g.	n.g.	n.g.	n.g.	n.g.	56	57
Prokop	1961a-b	II	199	59	44	59	102	90	345	68
Sondermeier and Flatow	1962	II	n.g.	n.g.	n.g.	n.g.	120	84	n.g.	n.g.
Thomas	1963	II	455	53	179	55	486	76	1120	63
Jakobowicz, Graydon and Simmons	1966	II	22	5	28	14	141	38	191	31
Boettcher	1967	II	406	48	110	60	418	85	934	66
Phansomboon	1968	II	48	42	80	62	88	82	216	66
Wilson and Green	1964	III	71	31	26	46	44	75	141	48
Hummel and Schöch	1967	III	43	44	40	30	51	86	134	57
		IV	43	100	40	100	51	100	134	100
Putkonen	1932	V	317	10	136	12	172	31	728	14

[a] I: no titration, with centrifugation; II: with titration, no reference to centrifugation; III: with titration and centrifugation; IV: multiple incubations; V: incomplete information about the method.

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

n.g. = information not given in the reference examined.

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involved the three blood groups, the order of decreasing presence of agglutinins is O, B and A.

Table II summarizes the data in relation to O individuals who showed agglutinins in their saliva. In our sample, both among MZ and DZ twins, the majority presented anti-A and anti-B, while there was equal probability of detecting only one of these

TABLE II

PRESENCE OF SALIVARY AGGLUTININS IN O INDIVIDUALS FROM DIFFERENT SERIES

Reference	No. studied	Percentage		
		With anti-A	With anti-B	With anti-A and anti-B
Sondermeier and Flatow 1962	61	7	8	85
Wilson and Green 1964	59	12	27	61
Boettcher 1967	355	13	10	77
Present communication [a]				
MZ	9	11	11	78
DZ	10	30	30	40
Total	19	21	21	58

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

χ^2 for heterogeneity among series: $\chi^2 = 20.0$, 6 *DF*, $P < 0.01$; excluding Wilson and Green's (1964) series: $\chi^2 = 6.5$, 4 *DF*, $P > 0.10$.

antibodies among them. Data from the other series are in general agreement with these findings, the exception being Wilson and Green's (1964) values, which show a greater frequency of persons with anti-B only when compared with those having solely anti-A.

A comparison about the presence of anti-B in the saliva of A_1 and A_2 persons from Porto Alegre and other places is given in Table III. The frequency observed by us in A individuals in general (24%) is lower than those obtained elsewhere. This frequency was similar in A_1 and A_2 subjects, in agreement with Thomas' (1963) sample; Prokop (1961*a*, *b*) and Boettcher (1967), however, have found a higher prevalence of agglutinins among A_2 persons.

Data about the discordance for the presence of ABO salivary agglutinins in the MZ and DZ twins studied in Porto Alegre are given in Table IV. When only one agglutinin can be present the amount of discordance was respectively 17% and 14% in MZ and DZ twins. In blood group O subjects there was about three times more discordance (MZ: 58%; DZ: 50%), a result which would be expected if this variable would depend to a certain extent on the number of sources of variation. The similarity of the results in MZ and DZ indicates that the role of inheritance in the variability of the trait in question must be low.

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TABLE III

PRESENCE OF ANTI-B IN THE SALIVA OF A_1 AND A_2 INDIVIDUALS FROM DIFFERENT SERIES

Reference	A_1		A_2		Total	
	No. studied	% with anti-B	No. studied	% with anti-B	No. studied	% with anti-B
Prokop 1961*a-b*	173	56	26	76	199	59
Thomas 1963	359	51	96	58	455	53
Boettcher 1967	315	44	91	63	406	48
Present communication[a]	16	25	5	20	21	24

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

TABLE IV

DISCORDANCE IN RELATION TO THE PRESENCE OF ANTI-A AND ANTI-B SALIVARY AGGLUTININS AMONG TWINS FROM PORTO ALEGRE

Blood groups and discordance in relation to anti-A and anti-B	Zygosity	
	MZ	DZ
BLOOD GROUPS A_1, A_2 AND B		
No. of pairs studied	18	7
No. discordant	3	1
Percentage	17	14
BLOOD GROUP O		
No. of pairs studied	12	10
Discordant for anti-A	2	0
Discordant for anti-B	3	3
Discordant for both	2	2
Total % of discordance	58	50
GENERAL TOTAL		
No. of pairs studied	30	17
No. discordant	10	6
Percentage	33	35

Table V compares the average plasma agglutinin titers observed in Porto Alegre with those obtained in two other series. The levels seen in MZ and DZ twins are not statistically different. The total averages encountered in our sample are similar to those observed in Sidney (except for A persons) and Freiburg (Hummel and Schöch 1967 — their results were not given in detail, so that they could not be incorporated in Table V); but they are significantly higher than those obtained in Tecumseh. Since our twins have all about the same age, this variable was not considered in the analysis; no sexual difference was detected by us in the distribution of these titers.

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TABLE V

PLASMA AGGLUTININ TITERS ($-1 \times \log_2$) IN SAMPLES FROM PORTO ALEGRE, TECUMSEH (USA) AND SIDNEY (AUSTRALIA)

Blood group	Agglutinin	Locality and nature of the sample		No. studied	Average titer	*SD*	Significance of the differences[a]
O	anti-A	P. Alegre[b]	MZ	20	6.6	1.50	—
			DZ	18	6.6	1.34	
			Total	38	6.6	1.41	
		Tecumseh[c]		1035	5.1	0.04	< 0.001
		Sidney[d]		47	7.1	1.74	> 0.10
O	anti-B	P. Alegre[b]	MZ	20	6.8	1.32	> 0.20
			DZ	18	7.3	1.13	
			Total	38	7.0	1.24	
		Tecumseh[c]		1035	4.0	0.04	< 0.001
		Sidney[d]		47	6.7	1.67	> 0.30
A	anti-B	P. Alegre[b]	MZ	20	6.6	1.67	> 0.80
			DZ	11	6.5	1.37	
			Total	31	6.6	1.54	
		Tecumseh[c]		144	3.7	0.12	< 0.001
		Sidney[d]		47	5.0	1.78	< 0.001
B	anti-A	P. Alegre[b]	MZ	7	6.1	0.69	—
			DZ	1	—	—	
			Total	8	6.0	0.76	
		Tecumseh[c]		87	4.0	0.11	< 0.001
		Sidney[d]		47	6.3	1.89	> 0.60

[a] Differences between types of twins and between Porto Alegre and the other cities.

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

[c] Unrelated individuals. Data from Grundbacher (1967). Cells suspended in saline.

[d] Unrelated individuals. Data from Ichikawa (1959). Cells suspended in AB serum.

The degree of genetic determination of the levels of these agglutinins was ascertained by the comparison of average titers (Table VI) and titration scores (Table VII) in the twins. As can be seen, despite the fact that the correlation coefficients were always higher among MZ, the h^2 estimates were not high, and the F ratio was significant in relation to the anti-B of A persons only.

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TABLE VI

DEGREE OF GENETIC DETERMINATION OF PLASMA AGGLUTININ LEVELS AS ASCERTAINED FROM THE AGGLUTINATION TITERS IN TWINS FROM PORTO ALEGRE

Blood group	Agglutinin	Zygosity	No. of pairs	Average intrapair difference	Variance		r	h^2	F
					Between	Within			
O	anti-A	MZ	20	0.8	3.01	0.42	0.75[c]	0.24	1.31
		DZ	18	0.8	2.47	0.56	0.63[b]		
O	anti-B	MZ	20	0.7	4.19	0.60	0.75[c]	0.36	1.57
		DZ	18	1.0	2.71	0.94	0.48[a]		
A	anti-B	MZ	20	0.5	4.73	0.30	0.88[c]	0.85	6.53[b]
		DZ	11	1.5	2.15	1.96	0.05		
B	anti-A	MZ	7	1.0	2.45	0.64	0.58	—	—
		DZ	1	—	—	—	—		

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.
[c] Significant at the 0.001 level.

TABLE VII

DEGREE OF GENETIC DETERMINATION OF PLASMA AGGLUTININ LEVELS AS ASCERTAINED FROM THE TITRATION SCORES IN TWINS FROM PORTO ALEGRE

Blood group	Agglutinin	Zygosity	No. of pairs	Average intrapair difference	Variance		r	h^2	F
					Between	Within			
O	anti-A	MZ	20	5.8	247.9	30.1	0.78[c]	0.29	1.41
		DZ	18	6.3	206.1	42.5	0.66[b]		
O	anti-B	MZ	20	7.8	353.3	47.5	0.76[c]	0.05	1.05
		DZ	18	7.9	326.3	49.9	0.74[c]		
A	anti-B	MZ	20	5.6	431.0	27.6	0.88[c]	0.76	4.19[a]
		DZ	11	11.1	186.4	115.8	0.23		
B	anti-A	MZ	7	7.9	247.8	38.6	0.73	—	—
		DZ	1	—	—	—	—		

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.
[c] Significant at the 0.001 level.

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DISCUSSION

The first question that has to be discussed in relation to the salivary agglutinins is related to the type of technique used by the different authors. They involved single or multiple incubation periods, methods with and without titration or centrifugation, while the ratio of the volume saliva/blood cells also varied. Therefore, the variability in the prevalence of saliva agglutinins observed in different series is not surprising. Superimposed on this source of variation we have the observed changes that occur in the levels of these substances if a given person is tested at different times (Nebert 1962, Otten 1966). Questions related to the formation of these agglutinins are of course also highly relevant to the problem of genetic determination. The evidence now favors the view that almost all of them are produced locally due to antigenic stimulation, and are not the result of selective transportation from the serum (Claman et al. 1967, Otten 1967, Bell and Fortwengler 1971).

At least two genetic hypotheses have been put forward to explain the presence of ABO agglutinins in saliva. Miyakoshi (1951) postulated a pair of recessive autosomic genes, while Schmitz-von Hülst and Clausnitzer (1960) suggested a dominant one. Other authors, however, showed that the available evidence was against a simple model of inheritance (Prokop 1961*b*, Wilson and Green 1964, Boettcher 1967). Our results, showing about the same degree of discordance for the presence of these substances in saliva of MZ and DZ twins, point in the same direction.

As for the plasma agglutinins, the levels observed in Porto Alegre are of the same order of magnitude in MZ and DZ twins, and our combined sample showed values similar to those obtained in two of three previous series. We conclude that the subjects we have studied probably constitute, for this characteristic, a random draw from the population of our city.

Using two methods of evaluating the levels of ABO plasma agglutinins we found correlation coefficients always higher among MZ, but only in persons of group A the F ratio was statistically significant. Nijenhuis and Bratlie (1962) also did not find higher intrapair differences in the titration scores of 24 same-sex DZ as compared to 71 MZ pairs of twins. These results are in conflict with those reported by Dahr (1941) for the agglutinin titers of 70 MZ and 73 DZ, since he obtained, for all comparisons, values which yielded F-ratios ranging from 3.4 to 6.8. Kalff and Hijmans (1969) also found a significant F in the analysis of IgM levels in 18 DZ and 45 MZ pairs. New studies are necessary to settle this question.

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RIASSUNTO

I livelli delle agglutinine plasmatiche ABO sono stati studiati in 47 coppie di gemelli MZ ed in 30 coppie di gemelli DZ con lo stesso gruppo sanguigno. Il grado di determinazione genetica di tale caratteristica, cosi come accertato dai titoli medi e dai livelli di titolazione, non sembra essere elevato. Benché i coefficienti di correlazione fossero sempre più bassi nei gemelli DZ, il rapporto *F* è risultato significativo solo in relazione all'anti-B di individui A. La presenza di questi anticorpi è stata anche ricercata nella saliva di 30 coppie MZ e 26 DZ. Essendo stato riscontrato più o meno lo stesso grado di discordanza, il ruolo dell'eredità deve essere basso per questo carattere.

RÉSUMÉ

Les niveaux des agglutinines plasmatiques ABO ont été étudiés chez 47 couples de jumeaux MZ et 30 couples de jumeaux DZ avec le même groupe sanguin. Sur la base des titres moyens et des niveaux de titulation, le degré de détermination génétique de ce caractère ne semble pas être élevé. Quoique les coefficients de corrélation soient toujours inférieurs chez les jumeaux DZ, le rapport *F* n'est significatif qu'en relation à l'anti-B chez les sujets A. La présence de ces anticorps a aussi été recherchée dans la salive de 30 couples MZ et 26 DZ. Plus ou moins le même degré de discordance ayant été trouvé, le rôle de l'hérédité dans ce caractère doit être limité.

ZUSAMMENFASSUNG

Bei 47 EZ- und bei 30 ZZ-Paaren gleicher Blutgruppe wurde der Gehalt an ABO-Agglutininen im Plasma untersucht. Nach den Durchschnittstitern und den Titrierungsniveaus zu urteilen, scheint die Erbbedingtheit dieses Merkmals nicht gross zu sein. Bei den ZZ waren die Korrelationskoeffizienten zwar immer niedriger als bei den EZ, doch war das Verhältnis *F* nur bei A-Individuen in Bezug auf Anti-B wesentlich. Bei 30 EZ und 26 ZZ wurde auch der Speichel auf das Vorhandensein dieser Antikörper untersucht. Nachdem auch hierbei mehr oder weniger der gleiche Diskordanzgrad gefunden wurde, dürfte der Erbeinfluss auf dieses Merkmal gering sein.

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