Streptococci, ABO Blood Groups, and Secretor Status

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A number of studies have been made correlating the incidence of various diseases with genetic polymorphisms in man. Although in most studies no significant association could be found, such studies eventually could throw some light on the biological significance of genetic polymorphism. Two relationships have proved significant in several studies: (1) between the incidence of rheumatic fever and/or rheumatic heart disease (RF/RHD) and blood group O, and (2) between this disease complex and ABH secretor status. In 15 of 16 studies on the association between rheumatic fever and blood O, there were more non-O affected persons than expected, although in most instances the deviation was not statistically significant (table 1). When pooled, the results do show a significant association between RF/RHD and non-O blood type. Similarly, in eight of nine studies on the association between RF/RHD and secretor status, there were more nonsecretors among affected persons than among controls, with two of the studies reporting a significant deviation (table 2).

Rheumatic fever and rheumatic heart disease are the result of infections with *Streptococcus pyogenes* group A. It seemed of interest, therefore, to search for a possible association between the presence of this organism, as tested by culturing from a throat swab, and the ABO and secretor status of these persons. The results of these studies will be presented here.

A few studies on ABO blood groups and other streptococcal diseases have been done. There are reports of three studies on scarlatina in the older literature (Körwer 1932; Nowak 1932; Brody et al. 1936). Two of these showed a relative deficiency of group O patients, but the differences are not significant. McCorkle (1962) found a relative deficiency of group O patients with streptococcal pharyngitis, but the difference from the controls is not significant in his study either.

If nonsecretors as well as non-O persons are more susceptible to streptococcal infections, this could be explained by some inhibitory effect of H substance on the growth of streptococci. Saliva is present at the spot where streptococci can cause pharyngitis. The mean titer of H substance in saliva of secretors with blood group O could be higher than in secretors with other blood groups of this system. This means that the mean inhibitory action of H substance could explain a relative deficiency of streptococcal diseases in secretors with blood group O, and thus of all persons with blood group O.

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MATERIALS AND METHODS

Patients, Carriers, and Controls

The first sample studied consisted of persons living in Voorhout. This is a village of about 5,000 inhabitants near Leiden. In this village a random sample was taken of 618 persons above the age of five years, using the registration of the total population in the municipal files. People living in a home for the aged or a boarding school and persons in military service were excluded, as they were living under epidemiological conditions different from the general population. The persons in the sample were examined three times, with three-week intervals, during the first months of 1963. The

TABLE 1

A SUMMARY OF STUDIES ON THE RELATIONSHIP BETWEEN ABO BLOOD GROUPS AND RF/RHD

INVESTIGATORS	GROUP	ABO TYPE		RELATIVE INCIDENCE*	χ^2
		O	Non-O		
Maxted 1940	RHD	94	106	1.07	0.214
	Control	2,137	2,251		
Walsh and Kooptzoff 1956	RF	110	149	1.09	0.381
	Control	500	623		
Addis 1959	RHD	280	258	1.08	0.726
	Control	3,177	2,721		
Clarke et al. 1960	RHD	105	158	1.44	8.254
	Control	7,842	8,215		
Glynn et al. 1959†	RF	255	354	1.14	2.438
	Control	6,775	8,271		
Hooper 1960	RHD	90	122	1.29	3.313
	Control	7,842	8,215		
Khattab and Ismail 1960	RHD	32	88	1.55	4.330
	Control	3,598	6,402		
Pham-Huu-Trung et al. 1961	RF	189	245	1.02	0.012
	Control	113	144		
Buckwalter et al. 1962	RF/RHD	301	451	1.22	7.068
	Control	22,392	27,587		
Dublin et al. 1964	RF	276	332	1.04	0.113
	Control	281	324		
Sartor and Fraser 1964	RHD	56	80	1.22	1.226
	Control	998	1,173		
Gershowitz and Neel 1965	RF	49	55	0.94	0.070
	Control	688	820		
Gualandri and Ballabio 1965	RHD	347	635	1.55	35.599
	Control	1,965	2,321		
Macafee 1965	RHD	203	228	1.37	10.472
	Control	15,714	12,852		
Zuber 1966	RF	15	45	1.48	1.726
	Control	13,200	26,800		
This report	RHD	151	209	1.18	1.998
	Control	666	779		

NOTE.—Statistical analysis using method of Woolf (1955):

	df	χ^2	P
Y	1	53.578	<.0005
Heterogeneity	15	24.237	.10–.20
Total	16	77.815	<.0005

* Relative incidence = RF/RHD (non-O:O) ÷ control (non-O:O).

† Quoted in Clarke et al. 1960.

completion rate was 82%–86%. On each occasion, throat swabs and blood samples were taken. Saliva was collected only once.

The patients with streptococcal pharyngitis came from the same village. The design of this part of the study has been described by Valkenburg et al. (1963).

The second population from which a sample was taken consisted of military recruits examined at two military training centers. The recruits came from all parts of the country. All recruits in two drafts were seen in the spring of 1962. Within three hours after their arrival in the center, the recruits had their throats swabbed, and one month later another throat swab was taken. Saliva was collected only once.

TABLE 2

A SUMMARY OF STUDIES ON THE RELATIONSHIP BETWEEN SECRETOR STATUS AND RF/RHD

INVESTIGATOR	GROUP	SECRETOR STATUS		RELATIVE INCIDENCE	χ^2
		Sec.	Nonsec.		
Glynn et al. 1959	RF	393	160	1.37	5.733
	Control	516	153		
Clarke et al. 1960	RHD	190	73	1.20	1.310
	Control	644	207		
Pham-Huu-Trung et al. 1961	RF	315	119	1.45	4.049
	Control	215	56		
Buckwalter et al. 1962	RF	279	97	1.16	1.198
	Control	971	290		
Dublin et al. 1964	RF	423	180	1.20	2.013
	Control	446	158		
Gershowitz and Neel 1965	RF	70	32	1.51	3.446
	Control	1,152	349		
Wan Ngo Lim et al. 1965	RF	69	35	1.16	0.250
	Control	73	32		
This report	RF	102	31	1.12	0.285
	Control	1,770	480		
	RHD	298	77	0.95	0.121
	Control	1,770	480		

NOTE.—Statistical analysis using method of Woolf (1955):

	df	χ^2	P
Y	1	12.301	.0010–.0005
Heterogeneity	8	6.104	.70 –.60
Total	9	18.405	.050 –.025

The third population consisted of schoolchildren. About 250 children between the ages of 6 and 12 years were examined one to six times at three-week intervals. The examinations were done at three elementary schools at Leiden in the winter of 1962 and the spring of 1963. The same children, excluding absentees and two children who received antibiotic therapy, were seen each time. Each time a throat swab was taken, and, except for the last time, saliva samples were collected.

The patients with rheumatic fever or acute nephritis had been admitted to a pediatric hospital because of their condition. They were ascertained through the clinical archives of these hospitals. For rheumatic fever, the modified Jones diagnostic criteria were used (American Heart Association 1955).

For the diagnosis of acute glomerulonephritis, a similar set of criteria was used.

Patients were accepted as having had acute hemorrhagic glomerulonephritis due to a preceding streptococcal infection if they had had two "major" criteria or one "major" criterion and two "minor" criteria. Major criteria were: (1) edema, especially around the eyes; (2) systolic blood pressure over 140 or diastolic over 100 mm; and (3) protein, erythrocytes, and cylindrical casts in the urine. Minor criteria were: (1) temperature over 38.5° C (101.3° F); (2) erythrocyte sedimentation rate of 20 mm or more after one hour; (3) 12,000 or more leukocytes per mm^3 of blood; (4) blood urea of 45 mg or more per 100 ml serum; and (5) a positive throat swab for group A streptococci or a significant change in antistreptolysin-O titer (ASO-titer).

The patients with mitral stenosis who were used in the study were all seen at the Department of Cardiology of the University Hospital at Leiden. They were only included after full cardiological examination, complete with heart catheterization. Commissurotomy for mitral stenosis was done at the Department of Thorax Surgery of the same hospital.

Bacteriological and Serological Methods

The methods used for identification of streptococci and determination of ASO-titers in sera are described elsewhere (Valkenburg et al. 1963; Goslings et al. 1963). ASO-titers are expressed in reciprocal values of the number of "quarter tubes" in a serial double dilution of sera: 20, 40, 80, 160 . . . are 4, 8, 12, 16 . . . "quarter tubes."

Dry cotton throat swabs were used. They were cultured on blood agar within four hours for the study at Voorhout. As all military recruits had to be swabbed in a few hours' time, culturing from their swabs had to be delayed. For this reason, charcoal impregnated swabs and a modified transport medium described by Stuart et al. (1954) were used. This medium contained 1% agar. The swabs were kept at 4° C for 1–20 days and cultured in fluid Pike's medium for 18–24 hr. Subsequently, the sediments were cultured and streptococci identified the same way as in the other parts of the study.

Culturing was done under relative anaerobic conditions by adding to each plate a small paper bag containing a freshly made mixture of pyrogallol, potassium bicarbonate, and infusorial earth. If large numbers of plates had to be cultured at the same time, they were put in glass jars through which a mixture of carbon monoxide and nitrogen was passed. Enclosed in these jars were bags with a similar freshly made mixture to maintain a semianaerobic atmosphere. Micrococci, when present, were subcultured on blood agar plates for 24 hr and left another day at room temperature to judge the color of the colony. All *Staphylococcus aureus* were tested for coagulase activity using the method of Cadness-Graves et al. (1943). If this gave a negative result, the strain was tested again with Fisk's method (1940). Only strains with coagulase activity were considered in the study. The methods for grouping and typing of streptococci have been described before (Valkenburg et al. 1963).

The throats of the schoolchildren and people in Voorhout were swabbed alternately, in sequence of arrival, by two experienced technicians. In each military center, all recruits were seen by one physician. Serum samples were kept at −20° C until all samples from one individual could be examined in the same series on the same day. The sera were marked in such a way that the technicians could not know which sam-

ples belonged to the same individual. One stock of streptolysin-O was used for all samples.

Secretor Status and H-Substance Titers

Saliva was collected in glass tubes and placed in boiling water for 10 min within two hours after collection. Each sample was centrifuged for 10 min at about 1,500 g. The supernatant was frozen at −20° C until tested. The secretor status was tested by the hemagglutination-inhibition method using an extract of *Ulex europeus*. For each set of tests, the *Ulex* extract was titrated with group O cells and diluted to a standard anti-H activity. Equal amounts of saliva and *Ulex* extract were mixed. After one hour at room temperature an equal amount of a 1% suspension of group O cells was added, and 20 min later the test was read. All tests were done with the same stock of *Ulex europeus* seeds and read by the same person. In 1,127 cases the reading could be compared with an earlier reading on a sample from the same individual. This showed different readings in 24 cases (2.1%). On retesting these 24 sample pairs, all but one showed similar results.

The same method was used for H-substance titration in secretor salivas. In this case, a serial double dilution of saliva was tested, and each time the *Ulex* extract was diluted until it gave a standard agglutination pattern with a dilution series of a control saliva. The same stock of red cells, kept at 4° C in Alsever's solution, was used for all tests. All tests were done within 12 days. Several controls were included in each test series. The saliva samples from carriers and those from noncarriers for streptococci were equally distributed over the test series, as were the blood groups of the donors.

Statistical Analysis

The χ^2 test for 2 × 2 tables is used without continuity correction (Yates), as this correction makes the test too conservative (Grizzle 1967). The method of Woolf (1955) is used to compare the results of several studies. Student's t test was used to compare series of titers.

RESULTS

Secretor Status

No significant difference in distribution of secretor status between the sexes or age groups was found in this study.

The relationship between secretor status and carrier* status for group A streptococci was tested in three independent samples: the sample taken at Voorhout, the military recruits, and the schoolchildren (table 3). Two of these three samples show a significant excess of carriers among nonsecretors. The smallest sample, the one of schoolchildren, does not, although five of six examinations of this sample show a trend in the same direction. The data of the second examinations of each sample are compared, as these are greatest in number. The combined data show a highly significant difference and an insignificant heterogeneity among the samples. Comparison of data from different examinations of one sample is not done, as these are not mutually independent.

* A carrier is defined as a person from whom group A streptococci were cultured.

TABLE 3

SECRETOR STATUS AND CARRIER STATUS IN GROUP A STREPTOCOCCI

GROUP	TOTAL NUMBER	CARRIERS		χ^2	P
		N	%		
	Voorhout				
First examination:					
Secretors	429	82	19.1	4.10	.050–.025
Nonsecretors	103	29	28.2		
Second examination:					
Secretors	438	58	13.2	5.87	.025–.010
Nonsecretors	106	24	22.6		
Third examination:					
Secretors	410	63	15.4	6.81	.010–.005
Nonsecretors	98	26	26.5		
	Military Recruits				
First examination:					
Secretors	1,079	103	9.5	4.17	.050–.025
Nonsecretors	301	41	13.6		
Second examination:					
Secretors	1,126	136	12.1	3.44	.10–.05
Nonsecretors	311	50	16.1		
	Schoolchildren				
First examination:					
Secretors	204	45	22.1	0.69	.50–.40
Nonsecretors	62	10	16.1		
Second examination:					
Secretors	209	40	19.1	1.36	.30–.20
Nonsecretors	63	17	27.0		
Third examination:					
Secretors	187	39	20.9	0.39	.60–.50
Nonsecretors	58	15	25.9		
Fourth examination:					
Secretors	181	41	22.7	0.14	.80–.70
Nonsecretors	53	14	26.4		
Fifth examination:					
Secretors	175	27	15.4	0.80	.40–.30
Nonsecretors	55	12	21.8		
Sixth examination:					
Secretors	184	21	11.4	0.02	.90–.80
Nonsecretors	58	7	12.1		

NOTE.—Statistical analysis using method of Woolf (1955) on data from second examinations of the three samples:

	x	χ^2
Voorhout	1.92	5.77
Military recruits	1.40	3.37
Schoolchildren	1.56	1.77

χ^2 analysis:

	df	χ^2	P
Y	1	9.92	<.005
Heterogeneity	1	0.99	.70–0.60
Total	2	10.91	<.050

A similar test for streptococci belonging to the other Lancefield groups did not show any consistency between the samples; no significant difference in carrier rate was found.

The difference in carrier rate for group A streptococci could be due to other bacteria competing with group A streptococci in the throats. For this reason, the relationship of staphylococcal carrier status to secretor status and to carrier status for group A streptococci was investigated in the sample from Voorhout. No consistent results were found in the three examinations of this sample, although during the second examination there is a barely significant deficiency among nonsecretors. The data did not show a significant relationship between the carrier rates for the two pyogenic cocci. Hence neither streptococci other than group A nor staphylococci were responsible for the relationship found for secretor status and group A streptococci.

Repeated examination of the schoolchildren was done to see if the difference in carrier rate was due to a higher acquisition rate or to a longer duration of the carrier status in nonsecretors (table 4). The longest uninterrupted series of examinations per

TABLE 4

DURATION OF THE CARRIER STATUS FOR *Streptococcus pyogenes* GROUP A IN SCHOOLCHILDREN

Number of Successive Examinations per Child (j)	Number of Successive Examinations in Which the Same Type of *Streptococcus pyogenes* Group A Was Cultured (x)							Average Number of Successive Positive Cultures in Carriers
	(i) 0	1	2	3	4	5	6	
	Secretors							
2	16	2	2	·	·	·	·	1.5
3	16	3	3	1	·	·	·	1.7
4	12	2	1	3	2	·	·	2.8
5	8	2	0	2	0	0	·	2.0
6	86	17	12	7	7	2	5	2.6
	Nonsecretors							
2	6	1	1	·	·	·	·	1.5
3	7	1	0	2	·	·	·	2.3
4	4	1	0	0	0	·	·	1.0
5	4	1	0	1	0	0	·	2.0
6	21	2	4	6	2	2	0	2.9
Weighted means:								
Secretors	.....	26.3	18.3	12.8	8.6	2.0	5.0	2.4
Nonsecretors	.....	6.6	4.8	8.5	2.0	2.0	0.0	2.5

NOTE.—Weighted means for secretors =

$$Y_{si.} = \sum_{j=2}^{6} \frac{(x_{s.j} + x_{n.j})x_{s..}}{x} \times \frac{x_{sij}}{x_{s\ j}};$$

for nonsecretors =

$$Y_{ni.} = \sum_{j=2}^{6} \frac{(x_{n.j} + x_{s.j})x_{n.}}{x} \times \frac{x_{nij}}{x_{n\ j}}.$$

child are included. Due to absences of children, the distribution of the lengths of these series are different for secretors and nonsecretors. To allow comparison the data are weighted for the lengths of the series. The duration of the carrier status among nonsecretors is a little longer than for secretors, but this is far from significant.

There is no indication of an ethnological difference between carriers and non-carriers. This makes stratification as a possible cause for the relationship very unlikely. The most likely explanation is a higher susceptibility for carrier status in nonsecretors. If this difference in susceptibility exists not only for carrier status but for streptococcal infections as well, a difference in incidence of late complications of these infections, such as rheumatic fever and rheumatic heart disease, could result. According to the definitions used in this study, it is the host's reactivity to streptococci which makes a carrier an infected person. Thus it is of interest to compare antibody reactions in secretors and nonsecretors. This was done on ASO-titers of 140 carriers from Voorhout. For secretors, a mean ASO-titer of 16.8 $\pm$ 3.8 quarter tubes was found, and for nonsecretors, 17.4 $\pm$ 3.3 quarter tubes. The difference is in the expected direction but far from significant ($t = 1.01$, df = 138, $.30 < P < .40$).

In the same village of Voorhout, patients with streptococcal pharyngitis were seen by their general practitioner. Due to the great number of criteria to fulfill to have this diagnosis accepted—one being a significant change in ASO-titer—only 62 patients were available. Fourteen (23%) are nonsecretors. This is more than could be expected from a sample of the same population (19%), but the difference is not significant. Another indication for the same relationship was found in the incidence of tonsillectomy in the population. Tonsillectomy is done for recurrent tonsillitis (not necessarily due to group A streptococci, but streptococci are the most frequent causative agents). From the 227 persons in the sample at Voorhout, 46 had had their tonsils removed. The number of nonsecretors among them was 16 (35%). This is a significant relative excess over the secretors, where 18% had had their tonsils removed ($.03 < P < .05$).

The rheumatic fever as well as the acute nephritis patients were seen in one of five pediatric hospitals in different parts of the country. This is why the total number of persons in the samples at Voorhout, the military recruits, and the schoolchildren was considered the best choice among the available controls. Thirty-one of 133 children who had had rheumatic fever were nonsecretors (23.3%). For the children who had had acute nephritis, these figures are 50 of 230 (21.7%). Both figures show a relative excess of nonsecretors compared with the controls (21.3%), but neither of the differences is significant.

The patients with mitral stenosis came from all over the country, and a small number are from outside the Netherlands. Here, also, the total number of persons in the samples at Voorhout, the military recruits, and the schoolchildren are the best controls available. These patients cannot be considered a random sample of patients in the Netherlands. Most of them were seen by cardiologists, who sent them to Leiden to find out if they should be operated for their valvular deformity. There is also a difference in the incidence of mitral incompetence between the sexes, which complicates the analysis of the data. Of the 257 patients with pure mitral stenosis, 167 are female (65%) and of the 39 patients with a combined mitral stenosis and incom-

petence, 16 are female (41%). This significant difference makes it necessary to analyze the data from each group separately.

Three hundred seventy-five patients with mitral stenosis were seen, 236 female and 139 male. Among the female patients, 54 are nonsecretors (22.9%), and among the male patients, 23 (16.5%), a difference which is not significant.

Mitral incompetence is a factor which adversely affects fitness for surgical therapy, and as fitness for surgical therapy certainly leads to a bias in ascertainment, these figures cannot be compared with those of the controls. To find out if this bias could have influenced the figures, patients with incompetence are compared with patients without incompetence (table 5). In this table, patients with mitral valve deformity only are included, since involvement of other valves, probably not due to rheumatic disease, can cause secondary mitral incompetence. This comparison shows that

TABLE 5

SECRETOR STATUS OF PATIENTS WITH MITRAL STENOSIS WHICH ARE NOT OPERATED

GROUP	TOTAL NUMBER	WITH MITRAL INCOMPETENCE		STATISTICAL TEST	P
		N	%		
Men:					
Secretors	30	10	33	Exact*	< .20
Nonsecretors	3	3	100		
Women:					
Secretors	32	5	16	Exact	< .002
Nonsecretors	13	9	69		
Men and women:					
Secretors	62	15	24	$\chi^2 = 14.71$	< .0005
Nonsecretors	16	12	75		

* Fisher's exact method for 2 × 2 contingency tables.

mitral incompetence is more common in nonsecretors. The difference is significant for women.

Thus not only sex but also secretor status seems to have a relationship with the kind of mitral valve disease found. This suggests that both factors have affected the way the host reacts to streptococcal infection with rheumatic heart disease.

Further evidence for this heterogeneity came from the surgeon's findings during operation for mitral stenosis. Before commissurotomy is done, he palpates the mitral valve with his finger, and in most cases he noted his impression of the degree of valvular "fibrosis" in the surgical record. This "fibrosis" is based only on the perception of a certain degree of stiffness by the surgeon's finger and does not necessarily stand for the histological term fibrosis. This kind of "fibrosis" was found relatively more often in secretors than in nonsecretors (table 6). It is not likely that the 32 patients whose degree of "fibrosis" was not noted would have made this relationship insignificant. If we assume their degree of "fibrosis" to be distributed in such a way that it decreases the significance of the whole group as far as possible, staying within

the 95% interval based on the figures from patients with known "fibrosis," the difference is still significant.

The presence of calcular calcification was also perceived by the surgeon's finger and noted in most cases. It was found relatively more often in nonsecretors, but the difference from the findings in secretors is not significant.

H-Substance Titers

The distinction between secretors and nonsecretors is made on the differences in H-substance titer in their salivas. This distinction will have limited value if the distributions of these titers in the two groups show an overlap. As no saliva sample with a titer of two units was found in this study, the distinction of the two phenotypes is

TABLE 6

SECRETOR STATUS OF PATIENTS WITH MITRAL STENOSIS, WITH OR WITHOUT VALVULAR "FIBROSIS"

GROUP	TOTAL NUMBER	WITH VALVULAR "FIBROSIS"		χ^2	P
		N	%		
Men:					
Secretors	59	53	90	4.37	.050–.025
Nonsecretors	12	8	67		
Women:					
Secretors	90	68	76	5.19	.050–.025
Nonsecretors	25	13	52		
Men and women:					
Secretors	149	121	81	9.65	.005–.001
Nonsecretors	37	21	57		

NOTE.—For 9 men (1 nonsecretor) and 23 women (2 nonsecretors) the degree of "fibrosis" was not noted.

fairly straightforward (fig. 1). The mean H-substance titer of secretors with blood group O ($\bar{x}_O = 8.8 \pm 2.1$) is significantly higher than the mean titer of secretors of any other group of the ABO system. The comparisons gave the following results: with group A: $\bar{x}_A = 8.1 \pm 2.5$, $t = 2.60$, df = 295, $P < .02$; with group B: $\bar{x}_B = 7.5 \pm 2.6$, $t = 3.28$, df = 192, $P < .005$; and with group AB: $\bar{x}_{AB} = 6.9 \pm 2.7$, $t = 3.13$, df = 166, $P < .005$. (This analysis assumes independence of the groups when in fact they are not.) The differences in mean titer between secretors of groups A, B, and AB are not significant.

If the probability of a positive throat swab is directly related to the quantity of H substance in saliva, a relationship with H-substance titer in secretors alone could be expected. Therefore, the H-substance titers in the saliva samples from military recruits were determined. There is a small difference in mean H-substance titer between carriers and noncarriers of group A streptococci, but this difference is far from significant (table 7).

Blood Groups

The most consistent of the relationships found in surveys on ABO blood groups and rheumatic fever or rheumatic heart disease is the one with blood group O. All studies except one showed a lower disease rate among patients with this blood group (table 1). The mitral stenosis patients seen at Leiden showed a similar relative deficiency of patients with blood group O. The difference with the controls is not significant, however, and a bias due to the ascertainment is possible. Nevertheless, the consistency of the results from the various studies makes the results rather convincing. The methods of ascertainment in these studies are very different. Some studies include only rheumatic fever patients diagnosed according to Jones's criteria; other studies include only mitral stenosis patients seen for surgery. A common factor in all these studies is the fact that the diseases can be considered to be complications of streptococcal infections. This makes a study of the relationship between blood group O and streptococcal carrier status a logical next step.

This study was done in two samples, the sample at Voorhout and the military

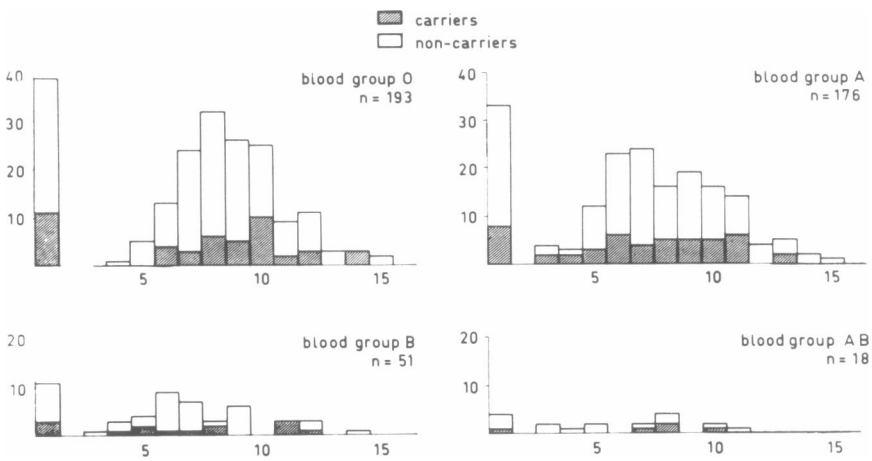

FIG. 1.—Distribution of H-substance titers in a population sample (Voorhout)

TABLE 7

H-SUBSTANCE TITERS WITH RESPECT TO SECRETOR STATUS AND CARRIER STATUS FOR *Streptococcus pyogenes* GROUP A

Blood Group and Carrier Status	Mean H-Substance Titer	*t*	df	*P*
O:				
Carriers	9.4±2.2	1.55	152	.20–.10
Noncarriers	8.6±2.1			
A, B, or AB:				
Carriers	8.0±2.6	0.60	195	.60–.50
Noncarriers	7.8±2.5			

recruits (table 8). Each time the sample at Voorhout was examined, a small difference in carrier rate was found, but none of these differences was significant. Both examinations of the military recruits revealed a relative excess of carriers with blood group O, one significant and the other not significant. These data do not fit in the general picture given by the other studies mentioned. There is no significant heterogeneity between the results of the second examinations of the sample at Voorhout and the military recruits. The results of this part of the study are inconclusive.

If a relationship were the result of a higher mean H-substance titer in salivas of secretors with blood group O, an association would be more evident in a study of secretors only. This was studied in secretors in both samples. The differences in carrier rate in secretors only were for each examination in the same direction as in the total samples, but none of these differences was significant.

The same argument holds for an association between blood group O and mitral stenosis. Here the deficiency of patients with blood group O was greater in secretors

TABLE 8

CARRIER STATUS FOR *Streptococcus pyogenes* GROUP A AND ABO BLOOD GROUPS

GROUP	TOTAL NUMBERS	CARRIERS		χ^2	P
		Number	%		
	Voorhout				
First examination:					
Blood group O.....	220	37	16.8	2.02	.20–.10
Blood group non-O..	283	62	21.9		
Second examination:					
Blood group O.....	238	41	17.2	1.18	.30–.20
Blood group non-O..	290	40	13.8		
Third examination:					
Blood group O.....	228	36	15.8	1.02	.40–.30
Blood group non-O..	270	52	19.3		
	Military Recruits				
First examination:					
Blood group O.....	614	71	11.6	8.64	.005–.001
Blood group non-O..	747	52	7.0		
Second examination:					
Blood group O.....	666	79	11.9	0.92	.40–.30
Blood group non-O..	779	80	10.3		

NOTE.—Statistical analysis using method of Woolf (1955) on data from second examination of the two samples:

	x	χ^2
Voorhout.....	1.30	1.18
Military recruits.....	1.18	0.96

χ^2 analysis:

	df	χ^2	P
Y.....	1	2.04	.20–.10
Heterogeneity.....	1	0.11	.80–.70
Total.....	2	2.15	.40–.30

than in nonsecretors (table 9). The difference in incidence of blood group O between male secretors and male nonsecretors is not significant however ($\chi^2 = 2.83$, df = 1, $.10 > P > .05$).

DISCUSSION

The assembling of adequate controls is one of the major problems in studies on the relationship between polymorphism and disease. It is hard to find adequate controls, especially for the mitral stenosis patients. The fact that each hospital has certain policies for admission of patients suggests controls selected according to similar policies, a requirement that is impossible to satisfy.

A common denominator to all samples of patients with rheumatic fever and/or rheumatic heart disease studied is the common origin of these diseases in streptococcal infection. The most frequent streptococcal infection is pharyngitis, and patients with streptococcal pharyngitis are usually not hospitalized for it. There still is a possibility of a serious bias in ascertaining streptococcal pharyngitis patients, as this is a self-limiting disease which gives only minor discomfort. In another study of the same patient population at Voorhout, it was found that probably most pharyngitis patients were not seen by their general practitioner and thus were missed in the survey.

The group of carriers for group A streptococci is the most unbiased group of this

TABLE 9

BLOOD GROUP O DISTRIBUTION IN PATIENTS WITH MITRAL STENOSIS

GROUP	TOTAL NUMBER	BLOOD GROUP O		χ^2	P
		N	%		
	Secretors Only				
Men	113	38	33.6	6.824	.010–.005
Women	175	81	46.3	0.002	.975–.950
Total	288	119	41.3	2.512	.20–.10
Controls	1,130	525	46.5	.....	.....
	Nonsecretors Only				
Men	23	12	52.2	1.265	.50–.40
Women	49	20	40.8	0.266	.70–.60
Total	72	32	44.4	0.002	.975–.950
Controls	315	141	44.8	.....	.....
	All Patients				
Men	136	50	36.8	4.319	.050–.025
Women	224	101	45.1	0.078	.80–.70
Total	360	151	41.9	1.998	.20–.10
Controls	1,445	666	46.1	.....	.....

NOTE.—The military recruits served as controls.

study and their controls are the most adequate. It is in this part of the study that the evidence for an association with nonsecretor of ABH substances in saliva is most convincing. This association alone could lead to a similar association with streptococcal pharyngitis and its complications, such as rheumatic fever and rheumatic heart disease. The heterogeneity between the sexes and different ways the rheumatic valvular disease presented itself during hospitalization make the action of additional selecting factors most likely. Only very few patients with endemic streptococcal pharyngitis in the general population will show signs of rheumatic heart disease, and this may be noted only many years after the initial infection. Selective factors associated with secretor status working in this period could very well exist. As long as the etiology of rheumatic heart disease is not fully understood, studies of selective factors cannot be conclusive.

A direct relationship between H-substance titer in saliva and carrier status for group A streptococci is possible. If this is the case, a lower carrier rate in persons with a high H-substance titer in their saliva can be expected. The mean H-substance titer in secretor carriers was higher than in secretor noncarriers, but this difference is not significant. The same relationship with H-substance titer can link the association with secretor status to the one with blood group O, as the mean titer in secretors with blood group O is higher than in non-O secretors. Although the many studies on ABO blood groups and rheumatic diseases are very convincing for an association between non-O and rheumatic disease, a similar association with the carrier status for group A streptococci was not found. We have tried to find an indication for a relationship between H-substance titer and streptococcal growth in in vitro studies but have not succeeded yet.

Family studies on streptococcal carrier status or streptococcal infections (e.g., to estimate heritability) are not rewarding. Not only do they show age dependency, the effect of crowding on the attack rate of streptococci gives intractable obstacles in building adequate models.

Since an association between streptococcal carrier status and mating type of the parents or an ethnic stratification are very unlikely causes for the association found, a gene-related susceptibility seems the most likely explanation. Streptococcal diseases are very rarely fatal diseases of childhood any more. They have declined in incidence and severity in most areas where figures for analysis are available (Stollerman et al. 1965; Findlay and Fowler 1966). In the last century and the early decades of this century, infant mortality from rheumatic carditis has been considerable, and a selective load due to this disease could very well have been substantial.

SUMMARY

Prompted by the results of various studies showing an association between nonsecretion of ABH substances and rheumatic fever, a similar study was done on the possible association between streptococcal carrier rate and pharyngitis. In several populations, a higher carrier rate for group A streptococci was found in nonsecretors. Although corresponding relationships with streptococcal pharyngitis, ASO-titer, acute nephritis, and rheumatic fever showed the same trend, none was significant

in our studies. The sample sizes in these studies were much smaller than the one in which the association with streptococcal carrier status was found, however.

As this association could be the result of a relationship between streptococcal attack rate and H-substance titer in saliva, the mean H-substance titer in secretor saliva of carriers was compared with the one in noncarriers. A nonsignificant difference in the expected direction was found.

Within the group of mitral stenosis patients, a significant heterogeneity in the distribution of secretors between the sexes was found. There also was a heterogeneity in secretor status between the patients subdivided according to complicating mitral incompetence and according to the degree of stiffness of the mitral valve during operation. It seems likely that secretor status is not only related to streptococcal carrier rate but also to the way some late complications of streptococcal infections present themselves.

For similar reasons, a relationship between ABO blood groups and streptococcal carrier status was studied. This study yielded no significant differences, however.

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