ON THE INHERITANCE OF AGGLUTINOGENS OF HUMAN BLOOD DEMONSTRABLE BY IMMUNE AGGLUTININS.[1]

By K. LANDSTEINER, M.D., AND PHILIP LEVINE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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Studies on the inheritance of serological properties were first undertaken systematically by von Dungern and Hirschfeld with the agglutinable substances in the blood of dogs (2) and with the human isoagglutinogens (3).[2] The authors named discovered the fact that the isoagglutinogens A and B are inherited as Mendelian dominants and this result has been amply confirmed by numerous workers.

According to their hypothesis there are two pairs of allelomorphic genes, *Aa*, and *Bb*, where *A* and *B*, the dominant genes, determine the presence of the corresponding agglutinogens, and *a* and *b*, the recessive genes, their absence. The genes for the blood groups are the following; group O:[3] aabb; group A: AAbb or Aabb; group B: aaBB or aaBb; group AB: AABB or AABb or AaBB or AaBb.

Another hypothesis has been advanced by Bernstein (6). He assumes multiple (three) allelomorphs, R, A, and B. The genetic formulæ accordingly are; group O: RR; group A: AA, AR; group B: BB, BR; group AB: AB. The theory of Bernstein does not involve a deviation from the older theory in the types of offspring except in the cases of parents belonging to group AB. According to the older view there may be children of any group in unions where one or both of the parents are in group AB; Bernstein's hypothesis, on the other hand, excludes children of groups O and AB in unions O × AB, and children O in unions A × AB, B × AB, or AB × AB. The recent work especially of Schiff (7), Thomsen (8), Preger (9), and Sievers (10) supports the opinion of Bernstein.[4, 5]

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[1] See the preliminary report (1).

[2] The problem of the inheritance of the human blood groups and a few results had been mentioned by Ottenberg and Epstein (4).

[3] The nomenclature of the blood groups by letters instead of numerals has been recommended both by the American Association of Immunologists and by the National Research Council (5) and is used in the present publication.

[4] The objection of Mendes-Correa (11) to the theory of Bernstein would imply

Tests for M and N in Several

Family No.	143						144					
Blood No.	F 298	M 299	300	301	302	303	F 304	M 305	306	307	308	
Group	A	A	A	A	A	A	O	O	O	O	O	
Reaction for M	+++	+++	++±	++±	+++	+++	++±	++±	++±	++±	+++	+
Reaction for N	−	−	−	−	−	−	+±	+±	+±	+±	−	

The strength of the reactions is indicated by the signs +, +±, ++, ++±, +++.

TABLE II.

Heredity of the Agglutinogen M.

Type of parents	No. of families	No. of children of type		Per cent of children of type	
		M+	M−	M+	M−
M+ × M+	101	403	33	92.4	7.6
M+ × M−	59	165	85	66.0	34.0
M− × M−	6	0	29	0	100

I.

F = Father; M = Mother.

145					146						147					
M 311	312	313	314	315	F 316	M 317	318	319	320	321	F 322	M 323	324	325	326	327
A	A	A	A	A	O	A	O	A	A	O	A	O	A	A	O	A
++	++	++±	−	++±	++	++±	++±	++±	++±	++±	−	++±	−	++±	−	−
+±	+	+	++	+±	−	++	+±	+±	−	−	++±	++	++±	+±	++±	++

TABLE III.

Heredity of the Agglutinogen N.

Type of parents	No. of families	No. of children of type		Per cent of children of type	
		N+	N−	N+	N−
N+ × N+	31	130	18	88.5	11.5
N+ × N−	29	81	40	66.9	33.1
N− × N−	4	0	17	0	100

The investigations outlined on the heredity of human blood groups are not only of theoretical interest[6] but they have attracted much attention because of their practical application in forensic medicine. A certain limitation lies in the fact that only two properties could be utilized. It is true that some experiments pointed to the existence of differences in human blood aside from the blood groups (13–16), but as a result of these studies no genetic investigations worthy of notice have been reported although obviously such would have been desirable. The reason for this is to be seen in the lack of workable methods.

The observations reported in previous publications (1, 17, 18) enabled us to undertake a study of the heredity of serological properties of human blood other than those determining the four blood groups.

With regard to the property designated as M there was no difficulty in selecting immune sera and absorbing bloods in such a manner that the reactions were either entirely negative on microscopic examination or so strong that clumps were visible to the naked eye.

The results with a second property, N, whose heredity was studied, varied depending upon the particular immune serum used. Although the strongest agglutinations occurred with the same bloods, there were differences in the reactions of minor strength so that the number of positive tests was greater with some sera than with others. In the following experiments two sera were selected which behaved identically and gave the smallest number of positive reactions; *i. e.*, the bloods acted upon by these sera reacted positively with all sera. Moreover with the sera chosen there was a distinct break between positive and negative tests, a point of significance for the present issue.

The frequency of the types M+ and M−, and N+ and N−, as already stated, is approximately the same in the four blood groups. According to our present results, there were in 1708 white individuals 326 (19.1 per cent) with negative reactions for M, and in 532 white individuals there were 139 (26.1 per cent) negative for N.

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that the formula $p + q + r = 1$, holds for arbitrarily chosen values, which is obviously not the case.

[5] While this paper was in press, another explanation based upon the assumption of incomplete linkage was proposed by Bauer (*Klin. Woch.*, 1928, vii, 1588).

[6] *Cf.* Morgan (12).

The technique of performing the tests has been described (18). The absorptions and tests for N were made at about 40°C.

It should be stated that the technique offers some difficulties as compared to the common isoagglutination tests. It is necessary to become well acquainted with the method and to know the properties of each serum in order to absorb completely all agglutinins but those in question.

The material for this study was obtained from two maternity clinics in the City of New York. Altogether 166 families were studied; in most of these (122) there were four or more children. Several families were always included in one experiment and also a number of control bloods with known properties.

TABLE IV.

Unions No.	Type of parents	No. of families	No. of children of types		
			M+N+	M+N−	M−N+
1	M+N+ × M+N+	11	31	17	7
2	M+N+ × M−N+	17	40	1	34
3	M+N+ × M+N−	24	60	40	3
4	M+N− × M−N+	5	17	0	1
5	M+N− × M+N−	4	0	17	0
6	M−N+ × M−N+	3 (6)	0	0	18 (29)

The figures in parentheses in unions of type 6, include the three families tested only for M but which, according to our experience, would be of the type M−N+.

In 166 families only the property M was investigated; 64 families were examined for M and N. A representative experiment is given in Table I.

The results for M are summarized in Table II and are arranged in three classes corresponding to the three types of unions and those for N in Table III are similarly arranged.

64 families were examined both for M and N. The results (Table IV) are arranged according to the six sorts of matings and the three types of offspring that have been observed.

A list of the tests is given in Tables V*a* and V*b* for the individual families, the former showing the tests for M (102 families) and the latter tests for both M and N (64 families). In each case the children are

TABLE V*a*.**

Reactions for M.

Family No.	Father	Mother	Children				
1	O+	B+	O+ ♂	O+ ♂	O+ ♀	O+ ♀	
2	O+	O+	O− ♀	O− ♂	O+ ♂		
3	AB−	AB+	AB− ♀	B+ ♂	B− ♂	A− ♀	
4	A+	A+	A+ ♂ A+ ♂	A+ ♀	A+ ♀	A− ♂	A− ♂
5	A+	A+	A+ ♀	A+ ♂	O+ ♀	A+ ♂	
6	B−	A+	B+ ♀	B+ ♀	B+ ♂	B+ ♂	
7	O+	O+	O+ ♂	O+ ♀	O+ ♂	O+ ♂	
8	AB+	A+	A+ ♂	B+ ♀	A+ ♂	A+ ♂	
9	A+	O−	A+ ♂ O+ ♀	O+ ♀	A+ ♀	O+ ♂	A+ ♀
10	B+	B+	O− ♂	B− ♀	B+ ♂		
11	O+	AB+	A− ♀	A+ ♂			
12	O+	A+	A+ ♀	O+ ♀	A+ ♀	A+ ♂	
13	O−	O−	O− ♀	O− ♀	O− ♀	O− ♀	
14	AB+	A+	A+ ♂	A+ ♂	A+ ♀	AB+ ♂	A+ ♂
15	AB+	A+	A+ ♀	AB+ ♀	AB+ ♂		
16	B+	O−	B− ♀	O− ♂	O+ ♀	O+ ♂	O− ♂
17	A+	O+	A+ ♂	O+ ♀	A+ ♂	O+ ♂	
18	A+	O+	O+ ♀	A+ ♀	A+ ♂*	A+ ♀*	O+ ♀
19	A+	O−	O+ ♀	O+ ♀	A+ ♀	O+ ♀	
20	A−	B+	O+ ♀	AB+ ♀	AB+ ♀	A− ♀	

* Twins.

** 20 of the 166 families examined were negro families.

TABLE V*a*—*Continued.*

Family No.	Father	Mother	Children				
21	A+	O+	A+ ♀	A+ ♀	O+ ♀	O+ ♂	
22	O+	O+	O+ ♀	O+ ♂			
23	O+	O+	O+ ♂	O+ ♂	O+ ♂	O+ ♂	O+ ♂
24	O+	O−	O+ ♂ O+ ♀	O− ♂ O+ ♀	O− ♂	O+ ♀	O+ ♂
25	B+	A−	AB− ♀ B+ ♀	B+ ♀	B+ ♂	AB+ ♀	AB+ ♂
26	AB+	A+	AB− ♂	A+ ♀	AB+ ♂	B+ ♀	B+ ♂
27	O−	A+	O− ♂	A+ ♀	A− ♂	A− ♂	
28	B+	O+	B+ ♂	O+ ♂	B+ ♀	O+ ♂	
29	O+	A−	A+ ♂	O− ♀	O− ♀	A+ ♀	O− ♀
30	O−	O+	O+ ♀	O− ♀			
31	A+	O−	A− ♀	O− ♀	A+ ♀	A+ ♀	
32	A+	O−	A− ♀	A+ ♀	A+ ♂		
33	O−	O+	O− ♂	O+ ♀	O− ♀	O− ♀	
34	B+	A+	A+ ♂	AB+ ♂	AB+ ♀	AB+ ♀	
35	A−	B+	A− ♀	AB− ♂	B− ♂	AB+ ♂	
36	O−	O+	O+ ♂	O+ ♂	O+ ♂	O+ ♂	O+ ♂
37	O+	A+	A+ ♀	A+ ♂	O+ ♀		
38	A+	A+	A+ ♂	O+ ♂	A+ ♂	O+ ♂	
39	O+	A−	A+ ♀	O+ ♂	O+ ♂		
40	O+	A+	A+ ♀	A+ ♀	A+ ♂	A+ ♂	A+ ♀
41	A+	B+	AB+ ♀ AB+ ♂	AB+ ♂	A+ ♀	AB+ ♀	AB+ ♂

TABLE V*a*—*Continued.*

Family No.	Father	Mother	Children				
42	A+	B−	A+ ♂	A− ♀	AB+ ♀	A− ♂	
43	A+	B+	A+ ♀	A+ ♂	AB+ ♀	A+ ♀	
44	B+	A+	A+ ♀	A+ ♂	A+ ♀		
45	B+	O−	O− ♂	B+ ♂			
46	AB−	O+	B+ ♀	B+ ♀*	B+ ♀*	B+ ♀	
47	A+	O+	O+ ♂	A+ ♀	O+ ♂	A+ ♂	O+ ♂
48	A+	A+	O+ ♂	O− ♂	O− ♂		
49	A+	A+	A+ ♀	A+ ♀	O− ♀	A− ♀	
50	B+	A+	B− ♀	O+ ♂	AB+ ♀		
51	O+	O−	O+ ♀	O+ ♀*	O+ ♀*	O+ ♀	
52	O+	O−	O+ ♂	O− ♀	O+ ♂	O− ♂	O− ♂
53	A+	B−	A+ ♀	AB+ ♂	AB− ♂	A− ♂	
54	A+	A+	A+ ♀	A+ ♂	A+ ♀		
55	A+	A+	A+ ♂	A+ ♀			
56	O+	A+	O+ ♂	A+ ♀	O+ ♀		
57	O−	O+	O+ ♀	O+ ♂	O+ ♀	O+ ♂	
58	O+	A+	A+ ♀	A+ ♂	A+ ♀		
59	O+	A+	O+ ♂	O− ♂	O+ ♂	O+ ♀	A+ ♂
60	B−	O−	O− ♂	O− ♂	O− ♀		
61	O+	A−	O+ ♀	O+ ♂	A+ ♀		
62	A+	A+	A+ ♀	O+ ♂	A+ ♂	O+ ♀	
63	O+	A+	O+ ♀	O+ ♂	A+ ♂	A+ ♂	A− ♂

* Twins.

TABLE V*a*—*Continued.*

Family No.	Father	Mother	Children				
64	O+	O+	O+ ♂ O+ ♂	O+ ♂	O+ ♀	O+ ♀	O+ ♂
65	O+	O−	O+ ♂	O+ ♀			
66	O+	B+	B+ ♂	B+ ♂			
67	A+	O+	A+ ♂	O+ ♂			
68	O+	O+	O+ ♀	O− ♂	O+ ♀	O− ♀	
69	A+	O+	O+ ♀	O+ ♀	A+ ♀	O− ♀	A+ ♂
70	A+	O+	O+ ♀ A+ ♂	A+ ♂ O+ ♂	O+ ♀ O+ ♂	O+ ♀ A+ ♀	O+ ♀
71	AB−	O+	O+ ♀ O+ ♀	A+ ♀	B+ ♂	A+ ♂	A+ ♀
72	B+	A−	O+ ♀	O+ ♀	B− ♀	AB+ ♀	
73	A+	O+	O+ ♀	O+ ♀	A− ♂	O+ ♂	
74	O+	A+	A+ ♀	A+ ♂	O+ ♂	O+ ♂	
75	B+	O+	O+ ♂	O+ ♂	B+ ♂	O+ ♀	
76	O+	A+	O+ ♂ O+ ♂	O+ ♂	A+ ♀	A+ ♂	A+ ♀
77	O−	B+	O+ ♂ O+ ♀	O+ ♀	O+ ♂	B+ ♀	O+ ♂
78	O+	O+	O+ ♀ O+ ♀	O+ ♂ O+ ♀	O+ ♂ O+ ♂	O+ ♀	O− ♀
79	O+	B+	B+ ♂	B+ ♂	B+ ♂		
80	O+	B+	O+ ♂	B+ ♀	O+ ♂	B+ ♂	O+ ♂
81	O−	B+	O+ ♀	O+ ♂	B+ ♂		
82	O+	O+	O+ ♂	O+ ♂	O+ ♂		

TABLE V*a*—*Concluded.*

Family No.	Father	Mother	Children				
83	O+	O+	O+ ♂	O− ♀	O+ ♀		
84	O+	O+	O− ♀	O− ♂	O+ ♂	O+ ♂	
85	O+	B−	B− ♂	B+ ♂	B− ♂		
86	A+	B+	B+ ♀	O+ ♀	AB+ ♀		
87	O+	A+	O+ ♀ O+ ♀	A+ ♀	O+ ♂	O− ♀	A+ ♂
88	O+	O−	O+ ♂	O+ ♀	O+ ♂		
89	A+	O−	A+ ♂ O+ ♀	O+ ♂	O+ ♂	A+ ♂	O+ ♂
90	AB−	O+	A− ♂	B− ♀	A+ ♂		
91	O−	AB+	A− ♂ B− ♀	B− ♀	A− ♀	B− ♀	A+ ♀
92	O+	O+	O+ ♀	O+ ♂	O+ ♂	O+ ♂	
93	B+	B+	B+ ♂	B+ ♂	B+ ♂	B+ ♂	
94	O+	A−	A+ ♀	A− ♂	A+ ♂	A− ♂	A+ ♀
95	B+	A+	B+ ♀	B+ ♂	O+ ♂	A+ ♀	
96	A+	A−	O− ♂ O− ♂	A− ♀	A+ ♂	A+ ♀	O− ♂
97	A+	O+	A+ ♂ O+ ♂	A+ ♀	O+ ♀	O+ ♀	O+ ♂
98	O+	A+	O+ ♀	O+ ♀	O+ ♀	O+ ♂	O+ ♀
99	B+	O+	B+ ♂ O+ ♂	B+ ♀	O+ ♀	O+ ♀	O+ ♂
100	A+	A+	A+ ♂	A+ ♂	A+ ♂	A+ ♀	
101	B+	O+	O+ ♂ B+ ♀	B+ ♂ O+ ♀	B+ ♀	O+ ♂	O+ ♂
102	B−	A−	A− ♀	A− ♀	A− ♂	AB− ♀	

TABLE V*b*.

Reactions for M and N.

Family No.	Father	Mother	Children			
103	O++	A++	A++ ♂	O+− ♀	A−+ ♂	O++ ♂
			A+− ♂			
104	O−+	A++	O−+ ♀	O++ ♂	O++ ♂	A−+ ♂
105	O−+	O−+	O−+ ♀	O−+ ♂	O−+ ♂	O−+ ♂
106	O++	AB+−	B++ ♂	B++ ♀		
107	O+−	A++	O+− ♀	A++ ♂	A+− ♀	A+− ♀
108	O+−	A+−	O+− ♀	A+− ♀	O+− ♂	O+− ♂
109	O+−	A++	A++ ♂	O++ ♂	A+− ♂	A+− ♂
			O−+ ♂			
110	A++	O−+	A++ ♀	O++ ♀	O++ ♂	O++ ♀
111	A+−	A−+	A++ ♀	A++ ♂	A++ ♀	A++ ♂
			A++ ♂			
112	O++	O+−	O++ ♂	O++ ♀	O++ ♀	O+− ♂
			O++ ♂			
113	A−+	A++	A++ ♂	A++ ♀	A−+ ♀	
114	A++	O++	A−+ ♀	O+− ♂	O−+ ♀	A++ ♀
			O+− ♂	A++ ♂		
115	A+−	A++	A++ ♂	A++ ♀	A++ ♀	A++ ♂
116	O++	B−+	B−+ ♀	O−+ ♀	B−+ ♂	O−+ ♂
			O++ ♂	B++ ♀		
117	O−+	O++	O++ ♀	O−+ ♀	O−+ ♀	O++ ♂
			O++ ♂	O++ ♂		
118	O+−	A++	O++ ♀	O++ ♂	O++ ♂	O+− ♀
			A+− ♀	A++ ♀	A+− ♀	O++ ♀
			A++ ♀			
119	O−+	A++	A−+ ♂	A−+ ♀	A++ ♀	O−+ ♀
			A++ ♂	A++ ♀	O++ ♀	

TABLE V*b*—*Continued.*

Family No.	Father	Mother	Children			
120	A++	O−+	A++ ♂	A++ ♀	A++ ♂	A++ ♀
121	B++	A++	AB−+ ♂	A++ ♂	A++ ♀	A++ ♂
			A+− ♂	A++ ♀		
122	O+−	A++	O++ ♂	A+− ♂	A+− ♂	A++ ♀
			A++ ♂			
123	O−+	A+−	A++ ♂	A++ ♀		
124	A+−	A++	A++ ♂	O+− ♀	A++ ♀	O+− ♂
			A+− ♀			
125	O++	A++	A+− ♂	A++ ♀	O++ ♀	A++ ♂
			O++ ♀			
126	B++	B++	B++ ♀	B+− ♂	O++ ♂	
127	A+−	B++	B−+ ♂	A+− ♀	O+− ♂	
128	O+−	O++	O+− ♀	O++ ♀	O+− ♀	O++ ♂
129	O++	O+−	O+− ♂	O+− ♂	O++ ♀	O++ ♀
			O+− ♀	O++ ♂		
130	A−+	A−+	A−+ ♂	O−+ ♂	A−+ ♀	A−+ ♀
			A−+ ♂	O−+ ♂		
131	A+−	O−+	O++ ♀	O−+ ♀	O++ ♂	O++ ♂
132	A−+	O++	A++ ♀	O−+ ♀	O−+ ♂	O++ ♂
			O++ ♂			
133	O++	O+−	O+− ♂	O++ ♀	O+− ♂	O+− ♀
			O+− ♀			
134	O+−	A+−	A+− ♀	A+− ♀	A+− ♂	
135	O+−	O++	O++ ♀	O+− ♂	O++ ♀	
136	A++	A−+	A−+ ♂	A−+ ♂	A−+ ♀	A−+ ♀
			O−+ ♀			
137	B+−	O++	B++ ♂	B+− ♀		

TABLE V*b*—*Continued.*

Family No.	Father	Mother	Children
138	A+−	O++	O++ ♀ A+− ♀
139	A+−	B−+	B++ ♀ B++ ♀ B++ ♀ AB++ ♀
140	A++	B++	O++ ♀ O++ ♂ A++ ♂ AB+− ♂ O++ ♀ O++ ♂
141	O++	B++	B++ ♀ B++ ♂ O++ ♀ O−+ ♂ O++ ♂
142	O+−	O++	O++ ♂ O++ ♂ O++ ♀ O++ ♂ O−+ ♂
143	A+−	A+−	A+− ♂ A+− ♀ A+− ♂ A+− ♂
144	O++	O++	O++ ♀ O++ ♀ O+− ♀ O++ ♂
145	O−+	A++	A++ ♂ A++ ♂ A−+ ♀ A++ ♀
146	O+−	A++	O++ ♀ A++ ♂ A+− ♂ O+− ♂
147	A−+	O++	A−+ ♀ A++ ♂ O−+ ♀ A−+ ♂
148	A++	B−+	B−+ ♀ A++ ♂
149	O−+	B−+	B−+ ♂ O−+ ♀ O−+ ♀ B−+ ♂ O−+ ♂ B−+ ♂ O−+ ♂ B−+ ♀
150	O−+	A++	A−+ ♀ A++ ♀ A+− ♂ A−+ ♀
151	B+−	O−+	O++ ♀ B++ ♀ B++ ♀
152	O−+	O++	O−+ ♂ O++ ♂
153	A−+	O++	A++ ♂ A−+ ♀ O−+ ♂ O−+ ♂ O++ ♀ O−+ ♀
154	A++	B+−	O++ ♀ B++ ♀ O+− ♂
155	A+−	A++	A++ ♂ A+− ♂ A++ ♂ A+− ♂ A+− ♂ A++ ♂
156	A++	O++	O++ ♀ O+− ♀ O+− ♂ A+− ♀

TABLE V*b*—*Concluded.*

Family No.	Father	Mother	Children			
157	O−+	O++	O++ ♀	O++ ♂	O−+ ♀	O−+ ♀
			O++ ♀	O−+ ♂		
158	O++	A−+	A++ ♀	A++ ♂	O++ ♂	
159	O++	B+−	B++ ♀	O++ ♀	B++ ♀	B++ ♂
160	A++	O++	A+− ♂	A++ ♀	O+− ♂	A−+ ♂
			O++ ♂	O+− ♀		
161	O++	A++	A++ ♀	A++ ♀	A+− ♀	A+− ♂
			A−+ ♀			
162	A+−	O++	A++ ♂	A++ ♂	A+− ♀	A+− ♂
			O++ ♀			
163	B+−	O+−	O+− ♂	B+− ♂	O+− ♂	B+− ♂
			O+− ♂	B+− ♂		
164	B+−	A++	O++ ♀	A++ ♂	A++ ♂	O++ ♂
			AB++ ♂			
165	B+−	O++	O++ ♀	O+− ♀	B+− ♀	O+− ♀
166	B+−	O++	B++ ♀	O++ ♀	B+− ♂	

recorded in order of decreasing age beginning with the eldest. The letters designate the groups, and the signs + and − the reactions for M (Table V*a*). In Table V*b* the first + or − sign designates the test for M and the second sign that for N.

As to the heredity of the factors A and B our results agree with the established fact that they are inherited as Mendelian dominants, except for three families in which A or B appeared in children when they were absent in the blood of the parents. These cases were considered as instances of illegitimacy and were excluded from the tabulations. One of these families was examined only for M and two for both M and N. The results were:

Father	Mother	Children			
O+	O+	O+♂	B+♂	O+♀	A+♀
O++	O++	A++♀	A++♀	O++♀	O+−♀
O+−	B−+	AB++♀	O++♂	O++♀	O++♀

In family 71, AB × O, there were two children in group O; the mother refused reexamination.

From the data reported it is evident that the agglutinogens studied are inherited properties. If we consider M and N separately they would seem to behave like Mendelian dominants. The characters cannot be recessive since in unions + × + there are children whose blood lacks the property. This result is to be expected if there are individuals among the parents heterozygous for a dominant character. If the absence of the agglutinogens is recessive there should occur no positive reactions in children from unions − × −. This is actually borne out by the observations. Thus in six such families with M− parents all the children (29 in number) gave negative reactions for M; likewise in the four families with N− parents all the children (17 in number) belonged to the N− type. In this respect our findings are analogous to the rule established by von Dungern and Hirschfeld for the isoagglutinogens A and B, *i. e.*, that these do not appear in the offspring if they are absent in both parents.

In order to discuss the numerical results for M alone in the three sorts of matings it is necessary to know the incidence of homozygous (MM) and heterozygous (Mm) individuals among the M+ parents. From a formula quoted by Johannsen (19), the following values are obtained: MM = 29.6; Mm = 49.6; mm = 20.8 (approximately 30, 50, 20, respectively). According to this formula the percentage of homozygous individuals equals $100 - 20\sqrt{R} + R$, that of heterozygous $20\sqrt{R} - 2R$; where R is the percentage of recessive (M−) individuals observed in the population. The figures of M+ and M− reactions are taken from all individuals, including the parents, of the 166 families examined for M.

Calculating from these figures the number of M− children in the unions M+ × M+ one has to consider only those in which both parents are heterozygous, *i. e.*, approximately 5/8 × 5/8; since 1/4 of the children of these matings should be M−, 9.8 per cent of M− children are to be expected; the observed value is 7.6 per cent.

In the unions M+ × M− 50 per cent of the offspring of heterozygous M+ parents may be expected to be M−; *i. e.*, 5/8 × 1/2 = 31.3 per cent; actually 34 per cent M− children were found.

In the smaller series where both factors were examined (see Table IV) the agreement between the calculated and the observed values is not so satisfactory.

Applying the formula of Johannsen for the factor N we obtain NN = 23.1; Nn = 49.8; nn = 27.1. Calculated as above the figures are in matings N+ × N+ 11.7 per cent N− children (observed 11.5 per cent) and in matings N+ × N− 34.2 per cent N− children (observed 33.1 per cent).

So far the cursory analysis of the results does not contradict the idea of two independent factors. However, there is evidence which does not seem compatible with this view. In the first place, if the factors were independent one would expect a certain, although small, percentage of bloods to lack both M and N, that is, if the genotype M−N− is not lethal, or its phenotype indistinguishable from one of the other types. In fact, as has been stated formerly (18) no such case has been found in the examination of more than 1200 specimens[7] and in each M− blood the reaction for N was found to be very strong. Further evidence emerges from an analysis of results in families examined for both properties. One sees that the frequency of M− children in the three sorts of matings, Nos. 1, 3, and 5 (Table IV) of parents M+, varies greatly according to the N reactions of the parents and that likewise the occurrence of children N− in matings 1, 2, and 6 is influenced by the presence or absence of M in the parents. A similar statement holds for the appearance of M− or N− children in matings 2 and 4, and 3 and 4, respectively.

Actually in most of the six sorts of unions the observed figures do not tally satisfactorily with those to be expected on the basis of two independent factors if one computes the expectancy from the figures given above for heterozygous and homozygous individuals. Thus in matings 2 and 3 there are too many children of the type M−N+ or M+N−, respectively, and in union 4 there appear with one exception only children of type M+N+. These numerical results could be interpreted in various ways. One hypothesis consists in assuming two genes which, when homozygous, would determine the phenotype M+N− and M−N+ respectively, while the phenotype M+N+

[7] This number includes the blood of negroes and Indians. About 900 of these were tested with the improved technique, namely, performing the tests for N at about 37–40°C.

would correspond to the heterozygous gene constitution. This view accounts for the non-existence of the type M−N−. On the basis of this hypothesis the expected values for the types of offspring are: mating 1: M+N+ 50 per cent, M+N− 25 per cent, M−N+ 25 per cent; mating 2: M+N+ 50 per cent, M−N+ 50 per cent; mating 3: M+N+ 50 per cent, M+N− 50 per cent; mating 4: M+N+ 100 per cent; mating 5: M+N− 100 per cent; mating 6: M−N+ 100 per cent.[8] Allowing for the relatively small number of individuals examined these figures agree fairly with those observed and better than the figures calculated for independent factors. Especially striking is the fact that in matings 2 and 3 there are almost no children M+N− or M−N+, respectively, and in mating 4 only one child not of the type M+N+. Still, there are five cases which contradict the hypothesis mentioned, namely, the individual M+N− in union 2, the three children M−N+ in union 3, and one child M−N+ in union 4. To attribute all these five exceptions to illegitimacy seems hazardous since only a proportion of illegitimate children would be detected by the tests employed and because the number of the exceptional cases is high compared with that of children which do not conform to the rule of von Dungern and Hirschfeld.

It may be pointed out that in all of the five exceptional cases in unions 2, 3, and 4, the father and not the mother is of the type opposite to that of the child, *e. g.*, father +−, child −+.

On the basis of the assumption just discussed and with the aid of the formula used above, the frequency of one type could be used to calculate the frequency of the other two types. Starting from the figure 20.8 for M− in a certain population the computation gave the value of 29.6 N−; similarly in a population of 205 Indians examined by us, the observed value of 4.9 M− leads to an expectancy of 60.7 for N−. Both these figures are in good agreement with those observed, namely, 26.1 and 60.0 respectively.[9]

An alternative hypothesis would suppose a close linkage between M and N. If, then, the gene combinations Mn and mN are numerically predominant this could explain the observed figures and also the occurrence of the exceptional cases aforementioned, but unless one assumes a lethal effect there arises a difficulty from the following con-

[8] The numbers of the matings refer to those given in Table IV.

[9] These results will be discussed in another publication (20).

sideration. If the factors M and N have been in the race for a long time the occurrence of cross-overs should by now have reduced the assumed numerical difference. However, on account of the existence of agglutinogens similar to M and N in anthropoids (chimpanzees) (18) it does not seem likely that they are due to recent mutations.

If in the unions listed as No. 1, Table IV, one of the parents be homozygous with respect to both M and N, then all children of such a union would be of the phenotype M+N+. The fact that this was not the case in any of the eleven families shows that none of the parents can be homozygous for both M and N, but such homozygous parents may have occurred in unions 2 and 3.

In view of the limited number of families studied it would seem premature to attempt a final interpretation and to discuss further possibilities such as the existence of more than two allelomorphs. Also it has to be considered that the state of affairs may be complicated, *e. g.*, by interacting or modifying effects of factors determining hitherto unknown agglutinable structures.

It may be added that there is no indication of a linkage between M and N and the isoagglutinogens A and B, and, as in the case of A and B, no evidence of a sex linkage.

SUMMARY.

The heredity of two agglutinable structures demonstrable by immune agglutinins was studied in 166 families. From the data collected it is evident that one deals with a case of Mendelian inheritance. The main result of the studies is the demonstration that it is feasible to investigate the heredity of serological structures of human blood other than the group agglutinogens. Irrespective of the ultimate theory it seems very probable that the properties M and N do not appear in the offspring when they are absent in both parents—a conclusion substantiated by the examination of ten families with 46 children. These findings offer the prospect of forensic application to cases of disputed paternity and, in our opinion, a correct decision could already be given, at least with great probability, provided the reagents are available and the method properly applied. Of course further work is needed before the test can be adopted as a routine procedure.

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