On the Serological Constitution of the Sei-, Fin-, Blue- and Humpback-Whales (I)

By

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Introduction

In 1901 Karl Landsteiner[1] classified the human blood groups by discovering the agglutinogens A and B in human erythrocytes according to the isohemagglutination. This blood grouping was named the "ABO System". In 1910–1911 von Dungern and Hirschfeld[2][3] confirmed that the blood group is a hereditary charactor. And then the trigemic theory was advanced by Bernstein[4] and Furuhata[5][6]. Thus the fundamental idea of blood groups was established. Furthermore, besides the classification by ABO system, such antigens as C[7][8], M, N[9][10][11][12][13][14][15][16], Q and Rh have come to be discovered. As for various mammals except human being the blood grouping by isohemagglutination, isohemolysis[17][18][19][20][21][22][23] and immune antibodies[24][25][26][27][28] have been worked out by many investigators. On the other hand by discussing on the distribution of the partial antigens[29][30][31][32][33][34][35][36][37] of each receptors in human erythrocytes, the problem of the systematic evolution of various animals was studied. Moreover the indexing of heterotype antigens[38][39][40] and the analysis of the structure of each type of receptors were worked out.

By these results the application scope of the blood groups covers on clinical medicine, medical jurisprudence[41][42][43][44][45], genetics[4][5][6][46][47][48][49][50] and anthropology[51][52][53][54].

The author, fixing attention on the said points, is working at the serological studies on whales. At first he tried to classify the antigens in each kind of whale erythrocytes, and he discovered the two antigens, namely Dc_1 and Dc_2, in the erythrocytes of the striped dolphin which belongs to the toothed whale. Consequently the dolphin bloods are classified into three kinds.[55] In the same manner as the above mentioned idea he immunized the rabbits with the erythrocytes of each kind of baleen whales, namely the sei-whale[56] caught near Bonin Islands, the fin-, blue- and humpback-whales of the northern Pacific Ocean. By thus obtained immune antibodies, he discovered the antigens Bb_1–Bb_2, Bp_1–Bp_2, Bm_1–Bm_2 and Mn_1–Mn_2 respectively. In consequence he was able to classify the whale bloods into the four kinds in each species respec-

tively.

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Material and Method

Erythrocytes and Serum of Whale: When a whale was pulled up to the working deck and its tail flukes were cut off, the blood which flowed out of the caudal artery was collected in a bottle. After coagulation, the erythrocytes were separated and cleaned with the physiological salt solution. These erythrocytes were used for immunization adsorption and reaction test. The separated serum was made inactive in the warm bath of 56°C for 30 minutes. And then the physiological salt solution with 5% carbolic acid was added, its quantity being 1/10 of that of the serum. After enough mixing it was preserved in the ice box.

Human Erythrocytes: The blood was taken from the elbow vein of a healthy person and was cleaned several times with the physiological salt solution and then centrifuged. The precipitated erythrocytes were used for adsorption, agglutination and hemolysis.

Immune Animal: The rabbit used for immunization must be healthy and 2.5 to 3.0 kg. in weight. Previous to immunization, its serum type and whether the rabbit is secretor or non-secretor concerning A receptor, namely A^+-type or A^--type was also examined.

Immunizing Method: At first the 10% suspension of the cleaned erythrocytes was made of the salt solution. Each other day 5 cc. of the suspension was injected into the ear vein of the rabbit, 7 time in all.

Collecting and Preserving Method of Antiserum: One week after the latest injection, the whole blood was collected from the carotid artery of the immunized rabbit. The separated serum was made inactive in the warm bath of 56°C for 30 minutes. And then the physiological salt solution with 5% carbolic acid was added to it, its quantity being 1/10 of that of the serum. After adequate mixing, it was preserved in the ice box. Food was withheld for 12 hours prior to the bleeding so as to prevent the turbidity of the serum.

Testing Method of Agglutination and Hemolysis: 30 minutes after mixing antigens and antibodies the agglutination was confirmed by the holeglass method in the room temperature. The hemolysis was judged by the test tube method, adding the guinea pig serum as complement, after 30 minutes warm bathing of 37°C.

Adsorption Test: Erythrocytes used for adsorption were to be the cleaned one, and to be regulated in quantity according to the dilution of the antiserum. After being left in the room temperature for a few hours the upper clear part was used for reaction. Meanwhile the mixture was shaken several times in order to make rapid the adsorption.

Isohemagglutination

(a) Sei-whale On rare occasions, the isohemagglutination was recognized in sei-whale, but its reaction was so weak that it was impossible to classify the antigens existing in the sei-whale erythrocytes. (See Table I)

Table I. Isohemagglutination of the sei whale.

		Erythrocytes of the sei whales															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Normal sera of the sei whales	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	11	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–
	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–

(b) Fin-whale The isohemagglutination was generally weak, but sometimes it was comparatively strong. In the latter case, some one showed a relation with the reaction by the immune agglutinin which is to be stated afterward. (See Sera Nos. 9 and 13 of Table II) But it was difficult to classify clearly the antigens existing in the erythrocytes. On the other hand the agglutinins which have no relation to the immune antibodies were found rarely. (See Sera Nos. 2, 3, 8 & 10 of Table II)

Table II. Isohemagglutination of the fin whale.

		Erythrocytes of the fin whales																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	20
Normal sera of the fin whales	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	−
	3	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
	4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	8	−	−	−	−	−	+	+	−	−	−	−	##	−	−	−	−	−	−
	9	−	##	−	−	−	+	+	−	−	−	+	##	−	−	−	−	−	−
	10	−	−	−	−	−	−	−	−	−	−	−	##	−	−	−	−	−	−
	11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	13	−	##	−	−	−	+	+	−	−	−	##	##	−	−	−	−	−	−
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

(c) Blue-whale Generally the isohemagglutination was comparatively strong, and showed a relation to the reaction with the immune agglutinin which is to be stated afterwards. It was possible to classify the antigens existing in the erythrocytes. (See Table III)

(d) Humpback-whale Generally the isohemagglutination was comparatively strong and showed a relation to the reaction with the immune agglutinin which will be described afterwards. The classification of the antigens which exist in the erythrocytes seems to be possible. (See Table IV)

As stated above, among the baleen whales the isohemagglutination was weak in the sei- and fin-whales, so it was impossible to classify the antigens existing in the erythrocytes by their reactions only. On the other hand isohemagglutination was found irregularly in the blue- and humpback-whales, but generally the reaction was strong, and it was possible to classify the antigens existing in the erythrocytes.

Table III. Isohemagglutination of the blue whale.

Normal sera of the blue whales	Erythrocytes of the blue whales											
	117	129	140	141	146	155	156	157	159	160	164	174
117	−	++	−	−	−	−	−	−	−	−	−	−
129	−	−	−	−	−	−	−	−	−	−	−	−
140	−	+	−	−	−	−	−	−	−	−	−	−
141	−	+	−	−	−	−	−	−	−	−	−	−
146	−	+	−	−	−	−	−	−	−	−	−	−
155	−	+	−	−	−	−	−	−	−	−	−	−
156	++	+++	−	−	−	−	−	−	−	+++	−	−
157	++	+++	−	−	−	−	−	−	−	+++	−	−
159	++	+++	−	−	−	−	−	−	−	++	−	−
160	−	+++	−	−	−	−	−	−	−	−	−	−
164	−	++	−	−	−	−	−	−	−	−	−	−
174	++	+++	−	−	−	−	−	−	−	+++	−	−

Table IV. Isohemagglutination of the humpback whale.

Normal sera of the humpback whales	Erythrocytes of the humpback whales									
	142	143	151	180	184	191	192	203	204	206
142	−	−	−	−	−	−	−	++	−	−
143	−	−	++	−	−	−	−	++	−	−
151	−	−	−	−	−	−	−	++	−	−
180	−	−	++	−	−	−	−	++	−	−
184	−	−	−	−	−	−	−	−	−	−
191	−	−	++	−	−	−	−	+++	−	−
192	−	−	++	−	−	−	−	+++	−	−
203	−	−	−	−	−	−	−	−	−	−
204	−	−	++	−	−	−	−	+++	−	−
206	−	−	−	−	−	−	−	+++	−	−

Serum-type

Whether the agglutinins against the receptors A and B of human erythrocytes exist or not in the normal sera of each whale species was examined by the agglutination to each type of human erythrocytes. The frequencies of these types were shown in Table V, in which it was known that o'- and α'-types are found in every whale species and the former has high frequency. The agglutinin titer of α'-type against human A erythrocytes was 2 to 4 times. On the other hand β'-type was found only in the two fin-whales (6.5%), namely Nos. 253 and 277. Their agglutinin titer against human B erythrocytes are 8 times. $\alpha'\beta'$-type was found in the fin- and blue-whales. As for the existence of anti-C agglutinin, the positive proof of the experiment of adsorption and dissociation by fresh human A and B type erythrocytes is not obtained yet.

Table V. Serum-type frequencies of the baleen whales.

(a) Sei whales

Serum-type	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
α′β′	0	0.0	0	0.0	0	0.0
α′	2	22.2	1	14.3	3	18.8
β′	0	0.0	0	0.0	0	0.0
o′	7	77.8	6	85.7	13	81.2
Total	9	100.0	7	100.0	16	100.0

(b) Fin whales

Serum-type	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
α′β′	3	18.8	0	0.0	3	9.6
α′	1	6.2	1	6.7	2	6.5
β′	0	0.0	2	13.3	2	6.5
o′	12	75.0	12	80.0	24	77.4
Total	16	100.0	15	100.0	31	100.0

(c) Blue whales

Serum-type	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
α′β′	5	21.8	2	14.2	7	8.9
α′	5	21.8	6	42.9	11	39.7
β′	0	0.0	0	0.0	0	0.0
o′	13	56.4	6	42.9	19	51.4
Total	23	100.0	14	100.0	37	100.0

(d) Humpback whales

Serum-type	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
α′β′	0	0.0	0	0.0	0	0.0
α′	2	25.0	4	57.2	6	40.0
β′	0	0.0	0	0.0	0	0.0
o′	6	75.0	3	42.8	9	60.0
Total	8	100.0	7	100.0	15	100.0

Antigens proved by immune antibodies

1. Immune antiserum against each antigen

(a) Anti-sei-whale Bb_1- and Bb_2-sera

When a rabbit was immunized with the sei-whale erythrocytes which belong to Bb_1, the anti-Bb_1 agglutinin and hemolysin were produced together with the species specific agglutinin and hemolysin to the sei-whale erythrocytes in the serum of the rabbit. When the species specific antibodies were adsorbed away with Bb_2 erythrocytes, the anti-Bb_1 immune agglutinin and hemolysin were obtained. The anti-Bb_2 immune agglutinin and hemolysin were obtained by the same operation. By these immune antibodies, it was proved that the existences of the both agglutinogens and hemolysinogens, namely Bb_1 and Bb_2 in both, were perfectly consistent with each other.

(b) Anti-fin-whale Bp_1- and Bp_2-sera

Anti-Bp_1 and anti-Bp_2 immune agglutinins and hemolysins were obtained by the same operation as stated in (a), using as antigens the fin-whale erythrocytes which belong to Bp_1 and Bp_2 respectively. By these immune antibodies it was proved that the existences of the both agglutinogens and hemolysinogens namely Bp_1 and Bp_2 in both, were perfectly consistent with each other.

(c) Anti-blue-whale Bm_1- and Bm_2-sera

Anti-Bm_1 and anti-Bm_2 immune agglutinins and hemolysins were obtained by the same operation as stated in (a), using as antigens the blue-whale erythrocytes which belong to Bm_1 and Bm_2 respectively. By these immune antibodies it was proved that the existences of the both agglutinogens and hemolysinogens, namely Bm_1 and Bm_2 in both, were perfectly consistent with each other.

(d) Anti-humpback-whale Mn_1- and Mn_2-sera

By the same method as stated in (a), the anti-Mn_1 and Mn_2 immune agglutinins and hemolysins against the humpback-whale erythrocytes were obtained. By these immune antibodies it was proved that the existences of the both agglutinogens and hemolysinogens, namely Mn_1 and Mn_2 in both, were perfectly consistent with each other.

As stated in (a) to (d), two kinds of antigens were found in each whale species, that is Bb_1-Bb_2, Bp_1-Bp_2, Bm_1-Bm_2 and Mn_1-Mn_2, in sei-, fin-, blue-and humpback-whales respectively. By the existences of these antigens the erythrocytes of the baleen whales were classified into each four kinds

as follows, Bb_1Bb_2, Bb_1, Bb_2 and O; Bp_1Bp_2, Bp_1, Bp_2 and O; Bm_1Bm_2, Bm_1, Bm_2 and O and Mn_1Mn_2, Mn_1, Mn_2 and O in the sei-, fin-, blue- and humpback-whales respectively.

2. Agglutinin titer and hemolysin titer

Each one example of the agglutinin titer and hemolysin titer of the immune sera obtained by the said method was to be shown in Table VI to IX.

Table VI. Agglutinin titer and hemolysin titer of the anti-Bb_1 and anti-Bb_2 immune sera against each type of the sei whale erythrocytes

Anti-Bb_1 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the sei whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 8 Male	o′	A+	No. 21 Bb_2	No. 13	Bb_1Bb_2	++++	++++	++++	++++	+	−	−	−
				No. 8	Bb_1	++++	++++	++++	+	+	−	−	−
				No. 21	Bb_2	−	−	−	−	−	−	−	−
				No. 6	O	−	−	−	−	−	−	−	−

Anti-Bb_2 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the sei whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 9 Male	α′	A+	No. 8 Bb_1	No. 13	Bb_1Bb_2	++++	++++	++++	++++	++++	+	−	−
				No. 11	Bb_1	−	−	−	−	−	−	−	−
				No. 21	Bb_2	++++	++++	++++	++++	++++	+	−	−
				No. 5	O	−	−	−	−	−	−	−	−

Anti-Bb_1 Hemolysin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the sei whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 8 Male	o′	A+	Bb_2	No. 13	Bb_1Bb_2	++	++	+	+	−	−	−	−
				No. 8	Bb_1	−	−	−	−	−	−	−	−
				No. 21	Bb_2	++	++	+	+	−	−	−	−
				No. 6	O	−	−	−	−	−	−	−	−

Anti-Bb_2 Hemolysim titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the sei whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 9 Male	α′	A+	Bb_1	No. 13	Bb_1Bb_2	++	++	+	+	+	−	−	−
				No. 11	Bb_1	−	−	−	−	−	−	−	−
				No. 21	Bb_2	++	++	+	+	+	−	−	−
				No. 5	O	−	−	−	−	−	−	−	−

Table VII. Agglutinin titer and hemolysin titer of the anti-Bp_1 and anti-Bp_2 immune sera against each type of the fin whale erythrocytes

Anti-Bp_1 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the fin whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 11 Male	o′	A+	Bp_2	No. 309	Bp_1Bp_2	++	++	++	+	+	−	−	−
				No. 310	Bp_1	++	++	+	+	+	−	−	−
				No. 316	Bp_2	−	−	−	−	−	−	−	−
				No. 308	O	−	−	−	−	−	−	−	−

Anti-Bp_2 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the fin whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 12 Male	α′β′	A+	Bp_1	No. 309	Bp_1Bp_2	++	++	++	++	++	+	+	−
				No. 310	Bp_1	−	−	−	−	−	−	−	−
				No. 316	Bp_2	++	++	++	++	+	+	+	−
				No. 308	O	−	−	−	−	−	−	−	−

Anti-Bp_1 Hemolysin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the fin whale	Dilution of antiserum							
No.	Serum-type	A^+ or A^-				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 11 Male	o′	A^+	No. 316 Bp_2	No. 309	Bp_1Bp_2	++	++	+	+	−	−	−	−
				No. 310	Bp_1	++	++	+	+	−	−	−	−
				No. 316	Bp_2	−	−	−	−	−	−	−	−
				No. 308	O	−	−	−	−	−	−	−	−

Anti-Bp_2 Hemolysin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the fin whale	Dilution of antiserum							
No.	Serum-type	A^+ or A^-				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 12 Male	α′β′	A^+	No. 310 Bp_1	No. 309	Bp_1Bp_2	++	++	++	++	+	+	−	−
				No. 310	Bp_1	−	−	−	−	−	−	−	−
				No. 316	Bp_2	++	++	++	+	+	+	−	−
				No. 308	O	−	−	−	−	−	−	−	−

Table VIII. Agglutinin titer and hemolysin titer of the anti-Bm_1 and anti-Bm_2 immune sera against each type of blue whale erythrocytes

Anti-Bm_1 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the blue whale	Dilution of antiserum							
No.	Serum-type	A^+ or A^-				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 13 Male	o′	A^+	No. 201 Bm_2	No. 297	Bm_1Bm_2	++	++	+	+	−	−	−	−
				No. 227	Bm_1	++	+	+	+	−	−	−	−
				No. 201	Bm_2	−	−	−	−	−	−	−	−
				No. 307	O	−	−	−	−	−	−	−	−

Anti-Bm_2 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the blue whale	Dilution of antiserum							
No.	Serum-type	A^+ or A^-				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 14 Female	α′	A^+	No. 227 Bm_1	No. 297	Bm_1Bm_2	++	++	++	++	++	+	+	−
				No. 227	Bm_1	−	−	−	−	−	−	−	−
				No. 201	Bm_2	++	++	++	+	+	+	−	−
				No. 307	O	−	−	−	−	−	−	−	−

Anti-Bm_1 Hemolysin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the blue whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 13 Female	α′	A+	No. 201 Bm_2	No. 297	Bm_1Bm_2	++	+	+	+	−	−	−	−
				No. 227	Bm_1	++	+	+	+	−	−	−	−
				No. 201	Bm_2	−	−	−	−	−	−	−	−
				No. 307	O	−	−	−	−	−	−	−	−

Ants-Bm_2 Hemolysin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the blue whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 14 Female	α′	A+	No. 227 Bm_1	No. 297	Bm_1Bm_2	++	++	++	++	+	+	−	−
				No. 227	Bm_1	−	−	−	−	−	−	−	−
				No. 201	Bm_2	++	++	++	+	+	+	−	−
				No. 307	O	−	−	−	−	−	−	−	−

Table IX. Agglutinin titer and hemolysin titer of the anti-Mn_1 and anti-Mn_2 immune sera against each type of the humpback whale erythrocytes

Anti-Mn_1 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the humpback whale	Dilution of antiserum							
No.	Serum. type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 15 Female	α′	A+	No. 211 Mn_2	No. 203	Mn_1Mn_2	++	++	++	+	+	+	+	−
				No. 151	Mn_1	++	++	++	+	+	+	+	−
				No. 211	Mn_2	−	−	−	−	−	−	−	−
				No. 206	O	−	−	−	−	−	−	−	−

Anti-Mn_2 Agglutinin titer

Immune rabbit			Erythrocytes for adsorp.	Erythrocytes for aggl.	Blood group of the humpback whale	Dilution of antiserum							
No.	Serum-type	A+ or A−				1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 16 Male	o′	A−	No. 151 Mn_1	No. 203	Mn_1Mn_2	++	++	++	++	+	+	−	−
				No. 151	Mn_1	−	−	−	−	−	−	−	−
				No. 211	Mn_2	++	++	++	+	+	+	−	−
				No. 206	O	−	−	−	−	−	−	−	−

Anti-Mn_1 Hemolysin titer

Immune rabbit No.	Serum-type	A^+ or A^+	Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the humpback whale	Dilution of antiserum 1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 15 Female	α'	A^+	No. 211 Mn_2	No. 203	Mn_1Mn_2	±±	±±	±±	±±	±	±	+	−
				No. 151	Mn_1	±±	±±	±±	±±	±±	±	+	−
				No. 211	Mn_2	−	−	−	−	−	−	−	−
				No. 206	O	−	−	−	−	−	−	−	−

Anti-Mn_2 Hemolysin titer

Immune rabbit No.	Serum-type	A^+ or A^-	Erythrocytes for adsorp.	Erythrocytes for hemoly.	Blood group of the humpback whale	Dilution of antiserum 1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
No. 16 Male	o'	A^-	No. 151 Mn_1	No. 203	Mn_1Mn_2	±±	±±	±±	±±	±	+	−	−
				No. 151	Mn_1	−	−	−	−	−	−	−	−
				No. 211	Mn_2	±±	±±	±±	±	±	+	−	−
				No. 206	O	−	−	−	−	−	−	−	−

3. Frequency of each type

As stated in the paragraph 1, the erythrocytes were classified into four kinds in each whale species. The frequencies of their groups were as follows. (see Table X) As shown in Table X (b), on fin whales were examined the 200 whales out of 213 which were caught in the northern Pacific Ocean in 1952. Consequently a remarkable seasonal variation was recognized in the frequency of each group. It seemed to the author that

Table X. Blood group frequencies of the baleen whales

(a) Sei whale

Blood group \ Sex	Male Actual No.	Male %	Female Actual No.	Female %	Total Actual No.	Total %
Bb_1Bb_2	4	18.2	2	20.0	6	18.8
Bb_1	4	18.2	3	30.0	7	21.9
Bb_2	2	9.1	1	10.0	3	9.3
O	12	54.5	4	40.0	16	50.0
Total	22	100.0	10	100.0	32	100.0

(b) Fin whale

Catch, during from July 19 to August 10

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Bp_1Bp_2	1	2.5	2	5.0	3	3.8
Bp_1	14	35.0	4	10.0	18	22.5
Bp_2	1	2.5	3	7.5	4	5.0
O	24	60.0	31	77.5	55	68.7
Total	40	100.0	40	100.0	80	100.0

Catch, during from August 11 to August 31

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Bp_1Bp_2	3	10.0	5	16.7	8	13.3
Bp_1	11	36.6	10	33.3	21	35.0
Bp_2	2	6.7	8	26.7	10	16.7
O	14	46.7	7	23.3	21	35.0
Total	30	100.0	30	100.0	60	100.0

Catch, during from Sept. 1 to Sept. 19

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Bp_1Bp_2	2	6.7	1	3.3	3	5.0
Bp_1	0	0.0	4	13.3	4	6.7
Bp_2	10	33.3	8	26.7	18	30.0
O	18	60.0	17	56.7	35	58.3
Total	30	100.0	30	100.0	60	100.0

Catch, during the whole season

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Bp_1Bp_2	6	6.0	8	8.0	14	7.0
Bp_1	25	25.0	18	18.0	43	21.5
Bp_2	13	13.0	19	19.0	32	16.0
O	56	56.0	55	55.0	111	55.5
Total	100	100.0	100	100.0	200	100.0

(c) Blue whale

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Bm_1Bm_2	1	3.3	1	5.9	2	4.3
Bm_1	8	26.7	7	41.1	15	31.9
Bm_2	4	13.3	1	5.9	5	10.6
O	17	56.7	8	47.1	25	53.2
Total	30	100.0	17	100.0	47	100.0

(d) Humpback whale

Blood group \ Sex	Male		Female		Total	
	Actual No.	%	Actual No.	%	Actual No.	%
Mn_1Mn_2	2	11.1	2	11.8	4	11.4
Mn_1	5	27.8	4	23.5	9	25.7
Mn_2	1	5.6	2	11.8	3	8.6
O	10	55.5	9	52.9	19	52.3
Total	18	100.0	17	100.0	35	100.0

it has relations to the problem of migration and mixing of the different population among whale races, judging from the fact that the frequencies of human blood groups show the different values in the different races. However, the detailed discussion on this will be made in another occasion. As for other species, such as sei-, blue- and humpback-whales, no particular tendency was found in consequence of scantiness of thecatch.

Isohemagglutinin against each antigens

As already stated isohemagglutination was recognized in the whale normal sera. In sei-whales, however, the agglutination was so weak and none reacts specifically to the antigens Bb_1 and Bb_2 proved by the immune antibodies. In normal sera of the fin-, blue- and humpback-whales exist the isohemagglutinins which are able to be completely adsorbed away with each antigen proved with the immune antibodies. But they were found irregularly. Their agglutinin titers were generally low in the fin-whale. In comparatively higher case they were 32 times at most. In the blue-whale they were slightly stronger, that is 64 times at most, than in the fin-whale. In some humpback-whales their titers came up to 128 times.

Some examples of isohemagglutinins against the antigens Bp_1, Bp_2: Bm_1, Bm_2 and Mn_1, Mn_2 of the fin-, blue and humpback-whales were shown in Table XI to XIII.

Table XI. Anti-Bp_1 and anti-Bp_2 isohemagglutinins proved in the normal sera of the fin whales

(a) Anti-Bp_1 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bp_1 agglutinin	216	188	Bp_1Bp_2	⧺	⧺	⧺	⧺	+	−	−	−
		193	Bp_1	⧺	⧺	+	−	−	−	−	−
		223	Bp_2	−	−	−	−	−	−	−	−
		221	O	−	−	−	−	−	−	−	−
	220	188	Bp_1Bp_2	⧺	⧺	+	+	−	−	−	−
		193	Bp_1	⧺	⧺	+	−	−	−	−	−
		223	Bp_2	−	−	−	−	−	−	−	−
		221	O	−	−	−	−	−	−	−	−
	221	188	Bp_1Bp_2	⧺	⧺	⧺	⧺	+	+	−	−
		193	Bp_1	⧺	⧺	+	+	−	−	−	−
		223	Bp_2	−	−	−	−	−	−	−	−
		221	O	−	−	−	−	−	−	−	−
	222	188	Bp_1Bp_2	⧺	⧺	⧺	+	−	−	−	−
		193	Bp_1	⧺	+	−	−	−	−	−	−
		223	Bp_2	−	−	−	−	−	−	−	−
		221	O	−	−	−	−	−	−	−	−
	223	188	Bp_1Bp_2	⧺	⧺	⧺	⧺	+	−	−	−
		193	Bp_1	⧺	⧺	+	−	−	−	−	−
		223	Bp_2	−	−	−	−	−	−	−	−
		221	O	−	−	−	−	−	−	−	−

(b) Anti-Bp_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bp_2 agglutinin	187	188	Bp_1Bp_2	++++	++++	+++	+	+	−	−	−
		165	Bp_1	−	−	−	−	−	−	−	−
		153	Bp_2	++++	++++	+++	+	−	−	−	−
		161	O	−	−	−	−	−	−	−	−
	246	188	Bp_1Bp_2	++++	+++	+++	+	−	−	−	−
		193	Bp_1	−	−	−	−	−	−	−	−
		223	Bp_2	++++	+++	+	+	−	−	−	−
		221	O	−	−	−	−	−	−	−	−
	247	188	Bp_1Bp_2	++++	++++	+++	+++	+	−	−	−
		193	Bp_1	−	−	−	−	−	−	−	−
		223	Bp_2	++++	++++	+++	+	+	−	−	−
		221	O	−	−	−	−	−	−	−	−
	249	188	Bp_1Bp_2	++++	+++	+	+	−	−	−	−
		193	Bp_1	−	−	−	−	−	−	−	−
		230	Bp_2	++++	+++	+	−	−	−	−	−
		231	O	−	−	−	−	−	−	−	−
	251	188	Bp_1Bp_2	+++	+++	+	−	−	−	−	−
		193	Bp_1	−	−	−	−	−	−	−	−
		230	Bp_2	+++	+	+	−	−	−	−	−
		231	O	−	−	−	−	−	−	−	−

(c) Anti-Bp_1Bp_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bp_1Bp_2 agglutinin	248	188	Bp_1Bp_2	++++	++++	++++	+++	+++	+	−	−
		193	Bp_1	++++	++++	++++	+++	+	+	−	−
		230	Bp_2	++++	++++	+++	+	+	−	−	−
		231	O	−	−	−	−	−	−	−	−
	253	188	Bp_1Bp_2	++++	++++	+++	+	−	−	−	−
		193	Bp_1	++++	+++	+	−	−	−	−	−
		230	Bp_2	++++	++++	+++	+	−	−	−	−
		231	O	−	−	−	−	−	−	−	−

Table XII. Anti-Bm_1 and anti-Bm_2 isohemagglutinins proved in the normal sera of the blue whales

(a) Anti-Bm_1 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bm_1 agglutinin	61	129	Bm_1Bm_2	++++	++++	+++	+++	++	+	+	+
		117	Bm_1	++++	++++	++	++	++	+	+	+
		160	Bm_2	+	+	+	+	+	+	+	+
		140	O	+	+	+	+	+	+	+	+
	155	129	Bm_1Bm_2	++++	++++	++	+	+	+	+	+
		117	Bm_1	+++	+++	++	+	+	+	+	+
		160	Bm_2	+	+	+	+	+	+	+	+
		140	O	+	+	+	+	+	+	+	+
	164	297	Bm_1Bm_2	++++	++++	++++	+++	+++	++	++	+
		227	Bm_1	++++	++++	+++	+++	++	+	+	+
		160	Bm_2	+	+	+	+	+	+	+	+
		159	O	+	+	+	+	+	+	+	+

(b) Anti-Bm_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bm_2 agglutinin	17	129	Bm_1Bm_1	++++	++++	++++	+++	++	+	+	+
		117	Bm_1	+	+	+	+	+	+	+	+
		160	Bm_2	++++	++++	+++	+++	++	+	+	+
		140	O	+	+	+	+	+	+	+	+
	157	129	Bm_1Bm_2	++++	++++	++++	++++	+++	+++	++	+
		117	Bm_1	+	+	+	+	+	+	+	+
		160	Bm_2	++++	++++	++++	++++	+++	++	++	+
		140	O	+	+	+	+	+	+	+	+
	205	297	Bm_1Bm_2	++++	++++	+++	++	+	+	+	+
		227	Bm_1	+	+	+	+	+	+	+	+
		199	Bm_2	++++	++++	+++	++	+	+	+	+
		196	O	+	+	+	+	+	+	+	+

(c) Anti-Bm_1Bm_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Bm_1Bm_2 agglutinin	156	129	Bm_1Bm_2	⧺	⧺	⧺	⧺	⧺	+	−	−
		117	Bm_1	⧺	⧺	+	+	+	−	−	−
		160	Bm_2	⧺	+	−	−	−	−	−	−
		140	O	−	−	−	−	−	−	−	−
	181	297	Bm_1Bm_2	⧺	⧺	⧺	⧺	⧺	+	−	−
		227	Bm_1	⧺	⧺	⧺	⧺	⧺	+	−	−
		160	Bm_2	⧺	⧺	⧺	⧺	+	−	−	−
		159	O	−	−	−	−	−	−	−	−

Table XIII. Anti-Mn_1 and anti-Mn_2 isohemagglutinins proved in the normal sera of the humpback whales

(a) Anti-Mn_1 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Mn_1 agglutinin	180	203	Mn_1Mn_2	⧺	⧺	⧺	⧺	⧺	⧺	+	+
		151	Mn_1	⧺	⧺	⧺	⧺	⧺	+	−	−
		211	Mn_2	−	−	−	−	−	−	−	−
		192	O	−	−	−	−	−	−	−	−
	191	203	Mn_1Mn_2	⧺	⧺	+	+	−	−	−	−
		151	Mn_1	⧺	+	−	−	−	−	−	−
		211	Mn_2	−	−	−	−	−	−	−	−
		192	O	−	−	−	−	−	−	−	−
	204	203	Mn_1Mn_2	⧺	⧺	⧺	⧺	⧺	⧺	+	−
		151	Mn_1	⧺	⧺	⧺	⧺	⧺	⧺	+	−
		211	Mn_2	−	−	−	−	−	−	−	−
		192	O	−	−	−	−	−	−	−	−

(b) Anti-Mn_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Mn_2 agglutinin	142	203	Mn_1Mn_2	++	++	++	+	−	−	−	−
		151	Mn_1	−	−	−	−	−	−	−	−
		211	Mn_2	++	++	+	+	−	−	−	−
		192	O	−	−	−	−	−	−	−	−
	151	203	Mn_1Mn_2	++	++	+	−	−	−	−	−
		151	Mn_1	−	−	−	−	−	−	−	−
		211	Mn_2	++	++	+	−	−	−	−	−
		192	O	−	−	−	−	−	−	−	−
	206	203	Mn_1Mn_2	++	++	++	++	+	−	−	−
		151	Mn_1	−	−	−	−	−	−	−	−
		211	Mn_2	++	++	++	+	+	−	−	−
		192	O	−	−	−	−	−	−	−	−

(b) Anti-Mn_1Mn_2 agglutinin

Normal sera		Erythrocytes for agglutination		Dilution of the normal sera							
Anti-body	No.	No.	Blood group	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Anti-Mn_1Mn_2 agglutinin	143	203	Mn_1Mn_2	++	++	++	++	+	−	−	−
		151	Mn_1	++	++	+	−	−	−	−	−
		211	Mn_2	++	++	++	++	+	−	−	−
		191	O	−	−	−	−	−	−	−	−
	192	203	Mn_1Mn_2	++	++	++	++	++	++	+	+
		151	Mn_1	++	++	++	++	++	+	−	−
		211	Mn_2	++	++	++	++	++	++	+	+
		191	O	−	−	−	−	−	−	−	−

Conclusion

(1) So far as just the author's survey goes, in the "serum type" of the four species of baleen whales, that is sei-, fin-, blue and humpback-whales, some

species have all of the four types, namely $\alpha'\beta'$, α', β' and o', while the other have only some part of the four. The frequencies of their types are as follows:

(a) Sei-whale $\alpha'\beta'$: 0.0%, α' : 18.8%, β' : 0.0%, o' : 81.2%
(b) Fin-whale $\alpha'\beta'$: 9.6%, α' : 6.5%, β' : 6.5%, o' : 77.4%
(c) Blue-whale $\alpha'\beta'$: 8.9%, α' : 39.7%, β' : 0.0%, o' : 51.4%
(d) Humpback-whale $\alpha'\beta'$: 0.0%, α' : 40.0%, β' : 0.0%, o' : 60.0%

(2) The existence of the two kinds of antigens, that is Bb_1-Bb_2, Bp_1-Bp_2, Bm_1-Bm_2 and Mn_1-Mn_2 in the erythrocytes of the sei-, fin-, blue- and humpback-whales respectively, were affirmed positively by the immune antibodies which were obtained by immunizing the rabbits with their erythrocytes. The erythrocytes of each species were able to be classified into four kinds as follows.

(a) Sei-whale Bb_1Bb_2: 18.8%, Bb_1: 21.9%, Bb_2: 9.3%, O: 50.0%
(b) Fin-whale Bp_1Bp_2: 7.0%, Bp_1: 21.5%, Bp_2: 16.0%, O: 55.5%
(c) Blue-whale Bm_1Bm_2: 4.3%, Bm_1: 31.9%, Bm_2: 10.6%, O: 53.2%
(d) Humpback-whale Mn_1Mn_2: 11.4%, Mn_1: 25.7%, Mn_2: 8.6%, O: 52.3%

(3) The frequency of each type of the fin-whales caught in the northern Pacific Ocean in 1952 showed a remarkable seasonal variation during the whaling season from July 19 to September 19.

(I) July 19 to August 10 (80 whales examined)
Bp_1Bp_2: 3.8%, Bp_1: 22.5%, Bp_2: 5.0%, O: 68.7%
(II) August 11 to August 31 (60 whales examined)
Bp_1Bp_2: 13.3%, Bp_1: 35.0%, Bp_2: 16.7%, O: 35.0%
(III) September 1 to September 19 (60 whales examined)
Bp_1Bp_2: 5.0%, Bp_1: 6.7%, Bp_2: 30.0%, O: 58.3%
Average through the whole season (200 whales examined)
Bp_1Bp_2: 7.0%, Bp_1: 21.5%, Bp_2: 16.0%, O: 55.5%

It seems that this fact can be taken for as the indicator of discriminating the local difference and mixing of different populations of whale races, judging from the meaning of biochemical racial index in human being. But detailed discussion must be looked for in future investigations. As for the other whale species no particular tendency was found because of the scantiness of the available number of whales.

(4) Isohemagglutinins was found in the normal sera on the four kinds of baleen whales. In the sei-whale it has no relation to Bb_1-Bb_2 system and the agglutinin titer was low. In the fin-, blue- and humpback-whales were found some agglutinins which react specifically to receptors of Bb_1-Bb_2, Bm_1-Bm_2 and Mn_1-Mn_2 systems respectively, and moreover their agglutinin titers were fairly high. But they were found irregularly.

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Errata for the Report: "On the Serological Constitution of Striped Dolphin (I)" inserted in the previous Bulletin No. 7

Page	Line	uncorrected	corrected
69	Contents 5	Existence	Detection
	〃 6	An Immune Antibody	The Immune Antibody
	〃 9	Appearance Rate	Frequency
	6-8	its range.....	its scope covers on partial antigens of A,
		.....	B, C [8)9)10)11)12)13)14)15)16)17)18)] O, M, N
		.....	and other receptors in the blood corpuscles
		.....in each charactor	of various animals
	14	D1 and D2	Dc1 and Dc2
	〃	from	by
70	18	coagulating reaction	agglutination reaction
	27	floating liquid	suspension
	〃	made with	made of
	28	5 cc of the liquid	5 cc. of the suspension
71	1	Coagulating Reaction	Agglutination Reaction
72	6	connected	concerned
	8	Existence	Detection
	12	coagulating reaction	agglutination
	Table 2	Absorption test	Adsorption test
	15	Type O′	Type o′
73	1	until the dilution with...	until the 4 times dilution with the salt
		.....in three times	water
	9	the agglutinin and.....	the species specific agglutinin and hemo-
		..... dolphin's blood	lysin
	12-15	From thus obtained.....	And then the former agglutinin and hemo-
		.....	lysin were adsorbed away by Dc2 blood
		.....	corpuscles from the antibodies. Conse-
		.....	quently, the anti-Dc1 immune agglutinin
		..by Dc2 blood corpuscle.	and hemolysin were obtained.
	16	immune serum	immune antisera
	17	agglutinins	agglutinogens
	18	hemolysins	hemolysinogens
	Table 3	each type of serum	each type of blood corpuscles
74	Table 4	each type of serum	each type of blood corpuscles
	1	Appearance Rate	Frequency
75	3	coagulating reaction	agglutination
	Table 5	coagulating reaction	agglutination
76	10	Dc1Dc2-type	Dc1Dc2-system
	13	appears	are found
	16	connection with Dc1Dc2 blood type	relation with Dc1Dc2 system
	19	O′	o′
	29-30	no connection with.....	no relation with Dc1Dc2-system, and they
		.....appear irregularly	were found irregularly.