

# On the Serological Constitution of the Sperm- and Baired beaked-Whales (I) Blood Groups of the Sperm- and Baired beaked-Whales

By

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## Introduction

In the serological studies on whales, the author at first tried to classify the antigens contained in their erythrocytes. Consequently it was found that four and three kinds of blood group exist in the sei-, fin-, blue- and humpback-whales<sup>1)</sup> belonging to the baleen whale and the dolphin<sup>2)</sup> belonging to the toothed whale respectively. In the same manner as in the above stated cases the blood groups of the sperm whale (*Physeter catodon*) and the baired beaked whale (*Berardius bairdii*) were classified into six kinds as the combination of the two systems, namely  $Pc_1Pc_2$  and Sp systems in the sperm and  $Br_1Br_2$  and Pb systems in the baired beaked respectively.

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## Material and method

*Erythrocytes of whale:* The coastal whaling grounds are generally far off and it takes comparatively long time to tow the catches to the whaling land station. So the carcasses are incised in the abdominal region to take away the blood for the purpose of keeping the meat fresh. By this reason it is difficult to collect the sera of whales. Whale erythrocytes were taken from the clots remaining in the heart and were cleaned with the physiological salt solution for the use of the immunization and the other reactions.

*Immune animal:* Domestic fowels were used as immune animal besides rabbits in the previous work, and prior to the immunization their serum-types were examined.

As regards human erythrocytes, immunization, antiserum, agglutination, hemolysis and adsorption test, materials and methods were just same as in the previous work.

### Isohemagglutination and serum-type

As the sera of whales had not been collected, the isohemagglutination and serum-type of whales were not examined.

### Antigens proved with immune antibodies

#### 1. Immune antiserum against each antigen

(a) Anti-sperm-whale  $Pc_1-$  and  $Pc_2-$  sera: When a rabbit or fowl is immunized with the sperm whale erythrocytes which belong to  $Pc_1Sp-$ , the anti- $Pc_1Sp-$  agglutinin and hemolysin are produced in the serum of the animal together with the species specific agglutinin and hemolysin against the sperm whale erythrocytes. When the species specific antibodies are adsorbed away from this serum with the  $Pc_2Sp-$  or  $Pc_1Sp-$  erythrocytes, the anti- $Pc_1$  immune agglutinin and hemolysin are obtained. The anti- $Pc_2$  immune agglutinin and hemolysin are obtained by the same operation. With these immune antibodies, it was proved that the existences of the agglutinogens and hemolysinogens, namely  $Pc_1$  and  $Pc_2$  in both, were perfectly consistent with each other. Consequently, the sperm whale blood was classified into three kinds, namely  $Pc_1Pc_2$ ,  $Pc_1$  and  $Pc_2$  groups.

(b) Anti-sperm-whale Sp-ferum: When a rabbit is immunized with such the  $Pc_1Sp-$  erythrocytes as contains Sp antigen, the anti- $Pc_1Sp-$  agglutinin and hemolysin are produced in the rabbit serum together with the species specific agglutinin and hemolysin against the sperm whale erythrocytes. If the species specific and the anti- $Pc$  antibodies are adsorbed away from its serum with the  $Pc_1Sp-$  erythrocytes, the anti-Sp antibodies are obtained. Instead of the  $Pc_1Sp+$  and  $Pc_1Sp-$ , using the  $Pc_2Sp+$  and  $Pc_2Sp-$  erythrocytes as antigens for immunization and adsorption respectively, the anti-Sp immune antibody may be also obtained. By this immune antibody the sperm whale blood was classified independently to the  $Pc_1Pc_2$  system into two groups, namely  $Sp+$  and  $Sp-$ : The former contains the Sp antigen and the latter doesn't contain it.

(c) Anti-baired beaked whale  $Br_1-$  and  $Br_2-$  sera: By the same method as described in the section (a), the existences of the  $Br_1$  and  $Br_2$  antigens which are contained correlatively each other in the beaked whale red cells were confirmed by the immune agglutinin and hemolysin. Consequently the blood groups of the beaked whale were classified into three kinds, namely  $Br_1Br_2$ ,  $Br_1$  and  $Br_2$  groups.

(d) Anti-baired beaked-whale Pb-serum: Methods in this section are just same as in the section (b). When an rabbit is immunized with the erythrocytes containing the Pb antigen, several kinds of antibodies are produced in the rabbit serum. If the anti-Br<sub>1</sub>, Br<sub>2</sub> and the species specific antibodies are adsorbed away from those by erythrocytes in which Pb antigen is not contained, anti-Pb antibody is obtained. By this immune antibody the baired beaked whale bloods are classified independently to the Br<sub>1</sub>Br<sub>2</sub> system, into the two groups, namely Pb+ and Pb-: The former contains Pb antigen and the latter doesn't.

## 2. Agglutinin titer and hemolysin titer

(a) Anti-sperm whale erythrocytes immune sera: Some examples of the agglutinin and hemolysin titer of the immune sera which is obtained by the said method are shown in the Tables I and II. It may be seen from these tables that the titer of the anti-Pc<sub>1</sub> antibody is lower in the case of using rabbit than fowl as immune animal. While it seems that the sensitivity in rabbit against Pc<sub>2</sub> is higher than that in fowl.

The titers of anti-Pc<sub>2</sub> antibodies which were produced in the rabbit serum by the four-time and seven-time (normal) immunization are shown in (b) of the Table I. It will be seen from the table that the titer of the anti-Pc<sub>2</sub> agglutinin already becomes up to 2,560 times after four-time immunization and reaches to such a high degree as 81,920 times by normal immunization. According to this fact, it seems to the author that the rabbit has a high sensitivity against Pc<sub>2</sub> antigen. As regards the difference between sensitivities of a rabbit against Pc<sub>1</sub> and those against Pc<sub>2</sub>, it is to be shown in (c) and (f) of Table I that the anti-Pc<sub>2</sub> antibody reached to a the higher titer than the anti-Pc<sub>1</sub> antibody when the rabbit is immunized with the mixture of erythrocytes of Pc<sub>1</sub>Sp- and Pc<sub>2</sub>Sp-. It will be seen from this fact that the rabbit seems to be more sensitive to Pc<sub>2</sub> than to Pc<sub>1</sub>.

In this experiment, for the purpose of comparison of the sensitivities against both antigens the erythrocytes which are used for the immune antigen should be made equivalently to the three individuals, in each type of Pc<sub>1</sub> and Pc<sub>2</sub>, and their both types of the erythrocytes had to be mixed equally in quantity. And then their total quantity was put into the 10% suspension of the salt solution and was used for the immunization by the regular method. The titers of the anti-Sp antibodies are shown in the Table II.

(b) Anti-baired beaked whale erythrocytes immune sera: Each one example of the agglutinin titer of the anti-bodies, which are produced

Table I. Agglutinin titer and hemolysin titer of the anti- $Pe_1$  and  $Pe_2$  immune sera

(a) Anti- $Pe_1$  agglutinin titer

Immune antigen	Immune animal		Erythrocytes for adsorption	Erythrocytes for agglutination		Dilution of antiserum															
	No., Sex	Serum type		No.,	Blood-group	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
$Pe_1$ Sp-	No. 26	o'	No. 7 $Pe_3$ Sp-	No. 12	$Pe_1Pe_3$ Sp-	##	##	##	+	-	-	-	-	-	-	-	-	-	-		
	Rabbit			No. 3	$Pe_1$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	
	Female			No. 9	$Pe_3$ Sp-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$Pe_1$ Sp-	No. 14	o'	No. 7 $Pe_3$ Sp-	No. 12	$Pe_1Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	
	Fowl			No. 3	$Pe_1$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
	Female			No. 9	$Pe_3$ Sp-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(b) Anti- $Pe_2$  agglutinin titer

Immune antigen	Immune animal		Erythrocytes for adsorption	Erythrocytes for agglutination		Dilution of antiserum																
	No., Sex	Serum type		No.,	Blood-group	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
$Pe_2$ Sp-	No. 23	α'	No. 3 $Pe_1$ Sp-	No. 12	$Pe_1Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##		
	Rabbit			No. 3	$Pe_3$ Sp-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Female			No. 9	$Pe_2$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
"	"	"	No. 3 $Pe_1$ Sp-	No. 12	$Pe_1Pe_3$ Sp-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	"			No. 9	$Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
	"			No. 12	$Pe_1Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
"	No. 15	o'	No. 3 $Pe_1$ Sp-	No. 12	$Pe_1Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	
	Fowl			No. 3	$Pe_1$ Sp-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Female			No. 7	$Pe_3$ Sp-	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##

\* This was collected in the next day of 4th immunization, namely 8 days after 1st immunization.  
 \*\* This was obtained by the normal immunization.















in the rabbit sera by immunization with each type of the beaked whale erythrocytes, are shown in the Tables III and IV.

It will be admitted from these tables that the rabbit is a little more sensitive to  $Br_1$  than to  $Br_2$ , but the difference of the sensitivities is not so remarkable as in case of the two antigens of the sperm whale.

In case of the immunization by the antigens  $Br_1$  and that by  $Br_2$  fowls as immune animal fell into convulsions and gave up their breath. So the antisera of the fowls were not obtained. It seems to the author that the baired beaked whale erythrocytes are poisonous to the fowl.

As shown in the Table IV, titers of the anti-Pb antibodies are not so high.

### 3. Frequency of each type

The frequencies of the blood groups which belong to the  $Pc_1Pc_2$  and Sp systems in the sperm whale and the  $Br_1Br_2$  and Pb systems in the beaked whale is shown in the Tables V and VI. As the sampling of these materials were not carried out systematically, it would be expected in the future work to discuss the problems on the whale resources and habits.

The relationship between the foetal blood group <sup>(3)</sup> and its mother whale's one is shown in the Table VI. The blood group of the foetus was confirmed with not only the agglutination and hemolysis but also with the adsorption test of the antibodies by their erythrocytes.

The body length at the period of delivery of the beaked whale foetus has not been classified enough up to the present day. From these examples, however, it may be approved that the blood groups are already formed at the body length of 2.53 meters (8 ft. 3 inch.) in male baired beaked whale and 2.61 metres (8 ft. 7 inch.) in female.

Table V Frequency of the blood groups of the sperm whale caught in the adjacent waters of Japan, 1953

Blood group Sex	Pc <sub>1</sub> Pc <sub>2</sub> -type			Pc <sub>1</sub> -type			Pc <sub>2</sub> -type			Total		
	Sp+	Sp-	To.	Sp+	Sp-	To.	Sp+	Sp-	To.	Sp+	Sp-	To.
Male	2	1	3	0	4	4	2	1	3	4	6	10
	(20.0)	(10.0)	(30.0)	(0.0)	(40.0)	(40.0)	(20.0)	(10.0)	(30.0)	(40.0)	(60.0)	(100.0%)
Female	3	2	5	3	2	5	2	4	6	8	8	16
	(18.8)	(12.5)	(31.3)	(18.8)	(12.5)	(31.3)	(12.5)	(24.9)	(37.4)	(50.0)	(50.0)	(100.0%)
Total	5	3	8	3	6	9	4	5	9	12	14	26
	(19.3)	(11.5)	(30.8)	(11.5)	(23.1)	(34.6)	(15.3)	(19.3)	(34.6)	(46.1)	(53.9)	(100.0%)

Table VI Frequency of the blood groups of the bairied beaked whale

(a) Catch off Ayukawa, Miyagi Prefecture in 1951. The classification of Pb system had not been performed yet.

Blood group Sex	Br <sub>1</sub> Br <sub>2</sub> -type		Br <sub>1</sub> -type		Br <sub>2</sub> -type		Total	
	Sp+	To.	Sp+	To.	Sp+	To.	Sp+	To.
Male	2	3	3	2	2	7	7	7
	(28.6)	(42.8)	(42.8)	(28.6)	(28.6)	(100.0%)	(100.0%)	(100.0%)
Female	1	2	2	1	1	4	4	4
	(25.0)	(50.0)	(50.0)	(25.0)	(25.0)	(100.0%)	(100.0%)	(100.0%)
Total	3	5	5	3	3	11	11	11
	(27.3)	(45.4)	(45.4)	(27.3)	(27.3)	(100.0%)	(100.0%)	(100.0%)

Table VI (cont.)

(b) Catch off Ayukawa, Miyagi Prefecture in 1953

Blood group Sex	Br <sub>1</sub> Br <sub>2</sub> -type			Br <sub>1</sub> -type			Br <sub>2</sub> -type			Total		
	Pb <sub>+</sub>	Pb <sub>-</sub>	To.	Pb <sub>+</sub>	Pb <sub>-</sub>	To.	Pb <sub>+</sub>	Pb <sub>-</sub>	To.	Pb <sub>+</sub>	Pb <sub>-</sub>	To.
Male	1	1	2	2	27	29	1	0	1	4	28	32
	(3.1)	(3.1)	(6.2)	(6.2)	(84.5)	(90.7)	(3.1)	(0.0)	(3.1)	(12.5)	(87.5)	(100.0%)
Female	0	0	0	0	7	7	0	1	1	0	8	8
	(0.0)	(0.0)	(0.0)	(0.0)	(87.5)	(87.5)	(0.0)	(12.5)	(12.5)	(0.0)	(100.0)	(100.0%)
Total	1	1	2	2	34	36	1	1	2	4	36	40
	(2.5)	(2.5)	(5.0)	(5.0)	(85.0)	(90.0)	(2.5)	(2.5)	(5.0)	(10.0)	(90.0)	(100.0%)

(c) Blood groups of the foetus

No.	Date, caught	Mother Whale			Foetus		
		Body length in ft.	Blood group	Sex	Body length in m.	Blood groups	Sex
7	July 14, '53	37	Br <sub>1</sub> Pb <sub>-</sub>	male	2.53	Br <sub>1</sub> Pb <sub>-</sub>	male
38	Aug. 6, '53	36	Br <sub>1</sub> Pb <sub>-</sub>	female	2.61	Br <sub>1</sub> Pb <sub>-</sub>	female

### Anti-Sp Heterohemagglutinin proved in the sei-whale normal serum

Heterohemagglutinin against the Sp antigen was found in the serum of No. 91 northern type sei-whale <sup>(4)</sup>(42 ft. long, male) having been caught in the northern Pacific Ocean, in 1953. The hemagglutination and adsorption tests by sperm whale erythrocytes are shown in the Tables VII and VIII. It may be admitted from this table that this agglutinin is completely adsorbed away by the Sp type cells in no connection with the Pc<sub>1</sub>Pc<sub>2</sub> system and is confirmed to be anti-Sp agglutinin. Its titer was about 8 or 16 times.

Table VII Agglutination of the anti-Sp agglutinin which was found in the No. 91 northern type sei-whale's serum against the each type of the sperm whale erythrocytes

Sperm whale erythrocytes		Dilution of the sei-whale serum							
No.	Blood group	$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$
10	Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	+++	+++	++	+	+	-	-	-
12	Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	+	-	-	-	-	-	-	-
5	Pc <sub>1</sub> Sp <sub>+</sub>	++	+++	++	+	-	-	-	-
3	Pc <sub>1</sub> Sp <sub>-</sub>	+	-	-	-	-	-	-	-
4	Pc <sub>2</sub> Sp <sub>+</sub>	++	+++	++	+	-	-	-	-
13	Pc <sub>2</sub> Sp <sub>-</sub>	+	-	-	-	-	-	-	-

Table VIII Adsorption test of the anti-Sp agglutinin which was found in the No. 91 northern type sei-whale's serum by the each type of the sperm whale erythrocytes

Sperm whale erythrocytes for adsorption	Sperm whale erythrocytes for agglutination	Dilution of the sei whale serum					
		$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$
No. 12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	+++	+++	++	+	+	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	++	+++	++	+	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	++	+++	++	+	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-

Table VIII (cont.)

Sperm whale erythrocytes for adsorption	Sperm whale erythrocytes for agglutination	Dilution of the sei whale serum					
		$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$
No. 3  Pc <sub>1</sub> Sp <sub>-</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	‡‡	‡‡	‡	+	+	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	‡	‡‡	‡	+	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	‡	‡‡	‡	+	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
No. 13  Pc <sub>2</sub> Sp <sub>-</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	‡‡	‡‡	‡	+	+	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	‡	‡‡	‡	+	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	‡	‡‡	‡	+	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
No. 10  Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
No. 5  Pc <sub>1</sub> Sp <sub>+</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
No. 4  Pc <sub>2</sub> Sp <sub>+</sub>	10 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	12 Pc <sub>1</sub> Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	5 Pc <sub>1</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	3 Pc <sub>1</sub> Sp <sub>-</sub>	-	-	-	-	-	-
	4 Pc <sub>2</sub> Sp <sub>+</sub>	-	-	-	-	-	-
	13 Pc <sub>2</sub> Sp <sub>-</sub>	-	-	-	-	-	-

### Conclusion

- (a) The existence of the two kinds of antigens and one antigen, which were found independently each other in the sperm whale erythrocytes, was affirmed positively by the immune antibodies obtained by

the immunization on rabbits or the domestic fowls with sperm whale red cells. In consequence, the sperm whale bloods were classified into six groups, namely  $Pc_1Pc_2SP+$ ,  $Pc_1Pc_2Sp-$ ,  $Pc_1SP+$ ,  $Pc_1Sp-$ ,  $Pc_2SP+$  and  $Pc_2Sp-$ .

(b) By the same manner as stated on sperm whale, baired beaked whale blood was classified into six groups, namely  $Br_1Br_2Pb+$ ,  $Br_1Br_2Pb-$ ,  $Br_1Pb+$ ,  $Br_1Pb-$ ,  $Br_2Pb+$  and  $Br_2Pb-$ .

2. (a) The frequency of blood groups of sperm whales caught in the adjacent waters of Japan is as follows:

$Pc_1Pc_2Sp+$ : 19.3%,  $Pc_1Sp+$ : 11.5%,  $Pc_2Sp+$ : 15.3%  
 $Pc_1Pc_2Sp-$ : 11.5%,  $Pc_1Sp-$ : 23.1%,  $Pc_2Sp-$ : 19.3%

(b) The frequency of blood groups of baired beaked whales caught off Ayukawa, Miyagi Prefecture, is as follows:

$Br_1Br_2Pb+$ : 2.5%,  $Br_1Pb+$ : 5.0%,  $Br_2Pb+$ : 2.5%  
 $Br_1Br_2Pb-$ : 2.5%,  $Br_1Pb-$ : 85.0%,  $Br_2Pb-$ : 2.5%

3. Two examples of blood groups of the baired beaked whale foetus were examined. Their blood groups were already formed at the body length of 2.53 meters in one male and at 2.61 metres in one female and were just same as their mother whales' blood group, namely  $Br_1Pb-$  type.

4. Heterohemagglutinin against the Sp antigen of the sperm whale red cells was recognized in the serum of the No. 91 northern type sei whale caught in the northern Pacific Ocean in 1953. Its agglutinin titer was about 8 or 16 times.

#### Literature

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