On the Serological Constitution of Striped Dolphin (*Prodelphinus caeruleo-albus* (Meyen)) (I)

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TABLE OF CONTENTS

Chapter 1. Introduction

Since Karl Landsteiner classified the human blood type according to isohaemagglutination at the beginning of the 20th century, the classification of blood type in various animals has been made according to isohaemagglutination,[1)2)3)4)5)] isohaemolysis and immune antibody.[6)7)] On the other hand, the advancement of serology has been so remarkable during the latest half century that its range covers the A, B, C,[8)9)] O, M, N of blood corpuscles in various animals and the distributive state of partial antigens[10)11)12)] in each character, up to the systematic evolution of animals and the indexing of heterotype antigens, and even to the analysis of the structure of each formal substance.

Serological study on whales which belong to the aquatic Mammalia has been hardly done up to the present. The authors, following the above stated results brought by the forerun researchers, have discovered the two antigens, namely D_1 and D_2, from the immune agglutinin and haemolysine produced by immunizing rabbits with the blood corpuscles of the striped dolphin, one of the aquatic mammals, and, by it, have been able to classify the blood corpuscle of striped dolphin into three kinds. So it is a great honor for them to report on it so as to receive a lot of precious critics from various worlds.

Chapter 2. Materials and Methods of the Experiment

Materials and methods of the experiment are to be summerized in the following. Details will be given in each clause.

Blood Corpuscle and Serum of Striped Dolphin

The spouting blood was taken into a pot when a dolphin had been pulled up to the operating place and his heart was stabbed with a knife. Blood corpuscles were separated from the blood clot on the absorbent cotton after coagulation by using physiological saline solution. After several times of centrifugal washing, the obtained blood corpuscles 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 saline solution with 5% carbolic acid was added to it. (Its quantity was 1/10 of that of the serum). After enough mixing, it has been preserved in the ice room.

Human Blood Corpuscle

A part of the blood taken, for the test of Wassermann's reaction, from the elbow veine of a healthy person was washed several times with physiological saline solution and was centrifuged. The precipitated blood corpuscles obtained thus were used for adsorption and coagulating reaction test.

Immune Animal

Serum type in the normal sera and whether the A character in the saliva was discharging type or non-discharging type, namely A+ type or A−type, were examined in a healthy rabbit, 2.5 to 3.0 kg in weight.

Immunizing Method

The blood corpuscles were washed several times with physiological saline water, and then the 10% floating liquid was made with saline water. Each 5 cc of the liquid was injected into the ear veine of a rabbit each other day. The total number of injection was 7.

Collecting and Preserving Method of Antiserum

One week after the last injection the whole blood was collected. The separated serum was made inactive in the warm bath of 56°C for 30 minutes. And then the physiological saline solution with 5% carbolic acid was added to it. (Its quantity was 1/10 of that of the serum). After adequate mixing, it has been preserved in the ice room. Any food had been given to the immune animal for about 12 hours before the blood collecting so as to prevent the turbidity of serum.

Testing Method of Coagulating Reaction and Hemolysis Reaction

30 minutes after, the coagulating reaction was judged by the holeglass method in the room temperature. The hemolysis reaction was judged by the test tube method, adding marmot serum as complement, after 30 minutes warm bathing of 37°C.

Adsorption Test

For adsorption, appropriate amount of the washed precipitated blood corpuscles had been mixed according to the dilution of antiserum. After being left in the room temperature for a few hours, the upper clear part was used for reaction.

Chapter 3. Isohaemagglutination

Isohaemagglutination in striped dolphin is generally weak. On rare occasions, however, it is comparatively strong, but not enough to

Table 1. Isohaemagglutination in *Prodelphinus caeruleo-albus* Meyen

Normal serum of striped dolphin (Serum) \ Blood corpuscle	Blood corpuscle of striped dolphin: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
1	– –
2	– –
3	– –
4	– – – – ++ – – – ++ – ++++ – – – ++ – – – – ++ – – – – – – – – – – – – – –
5	– –
6	– – – – – – + – + – – – – – – + – – – – – – – + – – – – + – – – – – –
7	– –
8	– –
9	– –
10	– –
11	– –
12	– –
13	– –
14	– –
15	– –
16	– – – – – – – ++ – ++ – – – – – – – – – – – – – – – – ++ – – – – – ++
17	– –
18	– –
19	– –
20	– –
21	– –
22	– –
23	– –
24	– –
25	– –
26	– –
27	– –
28	– –
29	– –
30	– –
31	– –
32	– –
33	– –
34	– –
35	– –
36	– –

classify easily the blood type with it. As shown in the Table 1, the reaction to the Sera No. 4 and No. 16 is comparatively strong, and moreover each agglutinin is adsorbed completely by each blood corpuscle which reacts positively to it so that the relation to the immune agglutinin which is to be stated after is noticed. On the other hand, the Serum No. 6 reacts weakly, but it seems not to be connected with what is to be stated after.

Chapter 4. Serum-type and the Existence of Anti C Agglutinin in Serum

Whether the agglutinins to the Human A, B and C characters exist or not in the normal serum of striped dolphin was examined by the coagulating reaction to each type of human blood. Some results of the reaction are given in the Table 2.

Table 2. Absorption test of striped dolphin's normal serum by Human Blood Corpuscles A, B and O

Serum / ** / *		Serum of striped dolphin																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Before absorption	A	++++	−	++	−	++++	−	−	−	−	++	−	+	++++	−	−	−	++++	−	++	−	−	−	−	−	−
	B	−	+	−	−	+	−	−	−	−	++	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−
	O	−	−	+	−	++	++	−	−	−	++	−	−	++++	−	−	−	++++	−	−	−	−	−	+	−	−
O	A	++++	−	−	−	++	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−
	B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A	A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B	A	++++	−	−	−	++	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−
	B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Work human blood corpuscle
** Absorption human blood corpuscle

As clarified in the above table, the Type α' is seen in the four dolphins (16%), namely Nos. 1, 5, 14 and 20, while the Type O′ in all the rest, that is 21 dolphins (84%). The Types β' and $\alpha'\beta'$ did not appear in any of the 25 dolphins. In the Type α', Nos. 1 and 5 react

positively until the dilution with the water whose quantity is three times as much as the original liquid, and Nos. 13 and 19 react positively only in the state of the original liquid. The existence of the normal anti C agglutinin was not testified in any serum.

Chapter 5. Antigens Dc_1 and Dc_2 Proved by An Immune Antibody

Paragraph 1. Anti Dc_1 Serum and Anti Dc_2 Serum

When a rabbit is immunized using as antigen the striped dolphin's blood corpuscle which belongs to Dc_1, the agglutinin and haemolysin, particular in each species, to the striped dolphin's blood corpuscle are produced in the serum of the rabbit. Simultaneously anti Dc_1 agglutinin and haemolysin are also produced. From thus obtained antibodies the anti Dc_1 immune agglutinin and haemolysin are obtained if the agglutinin and haemolysin, particular in each species, are adsorbed away by Dc_2 blood corpuscle. Anti Dc_2 immune agglutinin and haemolysin are obtained by the same operation. By the immune serum obtained by the above method, it was proved that the existences of both agglutinins and the both haemolysins, namely Dc_1 and Dc_2 were perfectly consistent each other.

Paragraph 2. Agglutinin Value and Haemolysin Value

Examples of the agglutinin value and haemolysine value of the anti Dc_1 and Dc_2 immune sera obtained by the method given in the previous Paragraph are shown in the Tables 3 and 4 respectively.

Table 3. Agglutinin value of anti Dc_1 and Dc_2 immune sera to each type of serum of striped dolphin

Anti Dc_1 agglutinin value

Immune rabbit			Absorption blood corpuscle	Work blood corpuscle	Blood type of striped dolphin	Dilution of antiserum					
Serum No.	Serum type	Existence or non-existence of Character A in saliva				1/20	1/40	1/80	1/160	1/320	1/640
No. 7 ♀	β′	O′	No. 8 Dc_2	No. 3	Dc_1 Dc_2	++++	++++	++++	+++	+	−
				No. 1	Dc_1	++++	++++	++++	+++	+	−
				No. 4	Dc_2	−	−	−	−	−	−

Anti Dc_2 agglutinin value											
Immune rabbit			Absorption blood corpuscle	Work blood corpuscle	Blood type of striped dolphin	Dilution of antiserum					
Serum No.	Serum type	Existence or non-existence of Character A in saliva				1/20	1/40	1/80	1/160	1/320	1/640
No. 6 ♂	o′	O′	No. 16 Dc_1	No. 3	Dc_1 Dc_2	++++	++++	++++	+++	+	−
				No. 1	Dc_1	−	−	−	−	−	−
				No. 4	Dc_2	++++	++++	+++	+++	+	−

Table 4. Haemolysine value of anti Dc_1 and Dc_2 immune sera to each type of serum of striped dolphin

Anti Dc_1 haemolysine value											
Immune rabbit			Absorption blood corpuscle	Work blood corpuscle	Blood type of striped dolphin	Dilution of antiserum					
Serum No.	Serum type	Existence or non-existence of Character A in saliva				1/20	1/40	1/80	1/160	1/320	1/640
No. 7 ♀	β′	O′	No. 8 Dc_2	No. 3	Dc_1 Dc_2	++++	++++	++++	+++	+	−
				No. 1	Dc_1	++++	++++	+++	+++	+	−
				No. 4	Dc_2	−	−	−	−	−	−

Anti Dc_2 haemolysine value											
Immune rabbit			Absorption blood corpuscle	Work blood corpuscle	Blood type of striped dolphin	Dilution of antiserum					
Serum No.	Serum type	Existence or non-existence of Character A in saliva				1/20	1/40	1/80	1/160	1/320	1/640
No. 6 ♂	o′	O′	No. 16 Dc_1	No. 3	Dc_1 Dc_2	++++	++++	++++	+++	+	−
				No. 1	Dc_1	−	−	−	−	−	−
				No. 4	Dc_2	++++	+++	+++	+++	+	−

Paragraph 3. Appearance Rate of Each Type

By the above stated method it has become clear that the two kinds, that is to say Dc_1 and Dc_2, of agglutinogen and haemolysinogen exist in the blood corpuscles of striped dolphin. The blood corpuscles of the 36 striped dolphins are classified by this into the following three kinds.

Blood type	Male	Female	Total
Dc_1 Dc_2	10 (50.0)	11 (68.8)	21 (58.3)
Dc_1	4 (20.0)	3 (18.7)	7 (19.4)
Dc_2	6 (30.0)	2 (12.5)	8 (22.3)
Total	20	16	36

(Parenthesized figure shows percentage.)

Chapter 6. Anti Dc_1 and Anti Dc_2 Isohaemagglutinin Seen in Normal Sera of Striped Dolphin

The Table 5 shows the respective comparison between the coagulating reaction of each type of the striped dolphin's blood corpuscles to

Table 5. Comparison between the coagulating reaction of each type of the striped dolphin's blood corpuscles to the anti Dc_1 and Dc_2 immune sera and the coagulating reaction to the normal sera Nos. 4, 16 and 6 of the striped dolphin which has the isohaemagglutinin.

	Work blood corpuscle / Abs. blood corpuscle	Blood corpuscles of striped dolphin													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Immune serum	Anti Dc_1 No. 4 (Dc_2)	±±±	±±	±±	−	±±	±±	−	−	±±	−	±±	±±	−	±±±
Immune serum	Anti Dc_1 No. 16 (Dc_2)	−	±±±	±±±	±±	−	±±±	±±±	±±±	−	±±±	−	±±±	±±±	±±±
	Blood type	Dc_1	Dc_1 Dc_2	Dc_1 Dc_2	Dc_2	Dc_1	Dc_1 Dc_2	Dc_2	Dc_2	Dc_1	Dc_2	Dc_1	Dc_1 Dc_2	Dc_2	Dc_1 Dc_2
Normal serum	Serum No. 4	−	−	−	−	±±±	−	−	−	±±	−	±±	±±±	−	−
Normal serum	SerumNo.16	−	−	−	−	−	−	−	±±±	−	±±±	−	−	−	−
Normal serum	Serum No. 6	−	−	−	−	−	−	+	−	+	−	−	−	−	−

	Blood corpuscles of striped dolphin																				
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Immune serum	±±	±±	±±±	±±±	±±	−	±±	±±±	±±±	±±±	±±±	±±±	±±	±±±	−	±±±	±±	±±±	±±±	±±±	±±	−
Immune serum	±±±	−	±±±	±±±	±±	±±	−	±±±	±±±	±±±	±±±	±±±	±±	±±	±±	±±±	±±	±±	±±	±±	−	±±
	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_2	Dc_1 Dc_2	Dc_1	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1 Dc_2	Dc_1	Dc_2
Normal serum	−	±±±	−	−	−	−	±±±	−	−	−	−	−	−	−	−	−	−	−	−	−	±±	−
Normal serum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±±	−	−	−	−	−	−	±±
Normal serum	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−

the anti Dc_1 and Dc_2 immune sera and the coagulating reaction to the normal sera Nos. 4, 16 and 6 of the striped dolphin which has the isohaemagglutinin.

According to this Table, the Serum No. 4 seems to react positively to all other Dc_1-type blood corpuscles than No. 1 and to have the anti Dc_1 normal agglutinin, but it reacts positively only to No. 13 and negatively to all others so far as Dc_1-Dc_2-type is concerned.

While the Serum No. 16 reacts positively only to the Blood corpuscles Nos. 8 and 10 of the Dc_2-type. The Serum No. 6 reacts so weakly that it has no relation to Dc_1-Dc_2-type at all.

That is to say that some normal isohaemagglutinins in the serum of striped dolphin show the similar reaction to the agglutinin obtained by immunization, but that their reaction is so weak and appears so irregularly that the blood type can not be classified clearly by this. On the other hand, there exist some other isohaemagglutinins whose reaction has no connection with Dc_1-Dc_2 blood type.

Chapter 7. Conclusion

1) So far as just our survey is concerned, the type α' and type O′ were found, but the type β' and type $\alpha'\beta''$ were not found in the serum of striped dolphin. The existence of anti C agglutinin was not testified in that serum.

2) The existence of the two kinds (Dc_1 and Dc_2) of antigen in the blood corpuscle of striped dolphin was testified by the anti Dc_1 and Dc_2 agglutinins and haemolysins obtained by immunizing the rabbit with the striped dolphin's blood corpuscle. The blood corpuscles of striped dolphin can be classified by this into the three kinds, namely Dc_1-Dc_2-type, Dc_1-type and D_2-type.

3) Some isohaemagglutinins of striped dolphin react particularly to Dc_1-type or Dc_2-type and some others react having no connection with Dc_1-D_2-type. It was found, but they appear irregularly.

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