ON THE SEROLOGICAL CONSTITUTION OF FIN WHALE
III. HUMAN B BLOOD GROUP SUBSTANCES IN ERYTHROCYTES AND SOME NOTES ON ANTI-FIN Ju SPECIFIC ANTIBODIES

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Many comparative studies have been undertaken on cell-antigens common to human and various kinds of animals. Especially, reports on distribution of the human A, B, O blood group substances and Forssman's antigen in animal kingdom, seem to be important and interesting from the standpoint of systematic serology. Terashima (1942) reports that human A blood group substance can be analyzed immunochemically into four partial antigens, i. e., AI, AII, AIII and AIV, and shows that A type human cells possess all of these, A type dog cells AII AIII AIV, A type pig cells AIII AIV and sheep cells AIV respectively. Friedenreich and With (1933) states that human B blood group substance may be divided serologically into three components, i. e., B_1, B_2 and B_3 and then the erythrocytes of B type human, rabbit and guinea pig contain $B_1B_2B_3$, B_2B_3 and B_3 respectively. As regards O antigen system Inoue (1943) describes that the erythrocytes of O type human, rat and rabbit possess OIOIIOIII, OIIOIII and OIII of the components of O blood group substances respectively. Basing upon these results many analysis have been worked out on partial antigens being contained in erythrocytes, saliva and other secretions or organ tissues of animals.

Symbols of Friedenreich and With have been used by some workers ever since. But in the present paper, to avoiding the confusion with those of B_1 and B_2 of human blood B subgroups, abovestated three groups are replaced with the symbols of BI, BII and BIII respectively in accordance with the proposal of Furuhata (1957). Summarizing the reports on B partial antigens in animal erythrocytes it is known that BIBIIBIII exist in the cells of human (Friedenreich and With, 1933), orang-utan (Dahr, 1937), monkey and ape (Noda, 1949) (Ishii et al, 1954), tortoise and frog (Sakuma, 1942), BIIBIII in the cells of rabbit (Friedenreich and With, 1933), Kangaroo, tapir, weasel, cat (Dahr, 1937) (Ogura, 1953a) and giraffe (Nakano, 1949) (Furuhata, Mori and Ro, 1949), BIII in the cells of guinea pig (Friedenreich and With, 1933), capuchin monkey, elephant, dog, badger, bison (Dahr, 1937), Japanese racoon-dog (Furuhata and Ro, 1948), camel and whales (AZAMI, 1949). Furthermore, Ogura (1953b) reports that the B substance in the horse and sheep erythrocytes has a simpler structure than BIII of guinea pig cells. Accordingly to the classification of

Friendenreich and With (1933) the author detects the B substances in the erythrocytes of whales, and confirmed the existence of some components of B antigen by adsorbing and immunizing experiments. The author reports here on a new B partial antigen in fin whale cells which has simpler structure than the already-known BIII in guinea pig cells, and simultaneously notes some observations on the anti-fin Ju normal antibodies in the sera of immune animals and the appearance of immune antibody into egg-white of a fowl.

HUMAN B BLOOD GROUP SUBSTANCES IN FIN WHALE ERYTHROCYTES

MATERIALS AND METHODS

Anti-BI, anti-BII and anti-BIII sera were prepared by the method of Yamaguchi (1943). Details of those and other items on materials and methods are as follows.

Anti-B_I agglutinin. When a rabbit is immunized with human B (BIBIIBIII) blood group erythrocytes, anti-BI antibody is produced alone in relation to B antigen, because the cells of rabbit possess BII and BIII components. This phenomenon is termed as the intravital filtration (Furuhata, 1957, p. 156). Then species-specific and anti-C agglutinins which are produced simultaneously should be adsorbed away from this serum by the A type human erythrocytes. These A type cells are taken from five or more individuals and are mixed together for use of adsorption. After these procedures may be obtained the anti-BI agglutinin available for the experiments.

Anti-B_{II} agglutinin. Being adsorbed the anti-C and the anti-O (Eisler's or Kagaya's anti-human cell heterogeneous antibody, (Eisler, 1930; Kagaya, 1940) agglutinins with A and O types human cells and anti-BIII agglutinin with guinea pig cells from the serum of fowl immunized with rabbit erythrocytes (BIIBIII), available anti-BII agglutinin can be obtained alone. A type cells which are used for adsorption are taken from several individuals similary to the previous case.

Anti-B_{III} agglutinin. If the anti-C and the anti-O agglutinins are adsorbed away from serum of a fowl immunized with the erythrocytes of guinea pig (BIII), the anti-BIII agglutinin will be taken out alone.

Erythrocytes of human and fin whales. Collecting, preserving and other treatments of these erythrocytes are just the same as those in previous report (Fujino, 1953).

Erythrocytes of rabbits and guinea pigs. Bloods were taken from ear vein in rabbit and from heart with syringe in guinea pig. After separation and washing with salt solution, these erythrocytes were used to various

experiments.

Immune animal. Mature rabbits and fowls, weighing more than 2.0 kg in the former and 1.5 kg in the latter, were used as immune animal. Prior to immunization, anti-human A and anti-human B normal antibodies, that is the "Serum type", were detected on the sera of these animals.

Immune sera. Each immune animal was given intravenously on alternate days a series of (seven times') injections of 10% erythrocytes suspensions. A dose of injection of these immune antigens is 5 ml. in a rabbit and 3 ml. in a fowl. Seven days after last injection, animals were sacrificed for bleeding by puncture of cervical artery. After separation and inactivation these immune sera (1) were added with 1/10 volume of 5% carbolic acid and preserved in refrigerator at the temperature of 0° to 5°C, or (2) were kept in freezer at the temperature of −5° to −10°C after mixing with 1/10 volume of 1% sodium azide (NaN_3).

Adsorption tests and immunizing experiments. For the purpose of detection of B antigen in the fin whale erythrocytes, adsorption tests and immunizing experiments must be made on their cells. Firstly the stated three partial antibodies were used in such adsorption tests. After thorough adsorption with fin whale cells, descendings of their titers against human B cells were examined. If the descent occurs, it is assumed that the fin whale cells used for adsorption possess the corresponding or simpler B antigen. When no descent occurs, existence of corresponding B antigen will be denied. Secondarily the existence of B antigen should be confirmed with immunizing experiments. Prior to immunization normal anti-human B antibody of immune animal (fowl) was examined. If acendings of titers of anti-human B agglutinins take place after immunization with fin cells, B antigen exists in their cells. In this case immunizing and post-immunizing procedures are just the same as those in preparation of anti-BIII agglutinin.

In case of fowls' immune sera, the cells of antigen show a tendency to cling on bottom wall of hole-glass before formation of agglutinating lumps, and therefore the more frequent shaking or mixing seem to be necessary during reacting period than in the case of rabbit sera.

EXPERIMENTS AND RESULTS

Preparation of anti-B_I, anti-B_{II} and anti-B_{III} sera. These partial antibodies were prepared with the method used by Yamaguchi (1943). Their titers did not generally reach up to so high, and are showed in table 1, i. e., 1 : 256 in anti-BI, 1 : 16 in anti-BII, and 1 : 256, 1 : 64 and 1 : 32 in anti-BIII agglutinins respectively. But these sera were useful enough to analysis of partial antigens.

TABLE 1. AGGLUTININ TITERS OF IMMUNE ANTI-BI, ANTI-BII AND ANTI-BIII SERA PREPARED FOR ADSORPTION TESTS

Name of serum	Immune animal	Normal sera of imm. animal: Treatment	Human cell	Dilution 1	2	4	8	16	32	64	128	Immune antigen	Immune sera: Treatment	Human cell	Dilution 1	2	4	8	16	32	64	128	256	512
Anti-BI no. 13 serum	No. 10 rabbit male (o')[4]	No ads.	A	—	—	—	—	—	—	—	—	Human B type cells	No[1] ads.	A	###	###	###	###	###	###	##	##	+	—
			B	—	—	—	—	—	—	—	—			B	###	###	###	###	###	###	##	##	+	—
			O	—	—	—	—	—	—	—	—			O	###	###	###	###	###	##	##	+	—	—
													Ads.[3] by human A cells.	A	—	—	—	—	—	—	—	—	—	—
														B	###	###	###	###	###	###	##	+	+	—
														O	—	—	—	—	—	—	—	—	—	—
Anti-BII no. 45 serum	No. 18 fowl female ($\alpha'\beta'$)	No ads.	A	###	##	##	+	—	—	—	—	Rabbit cells	No [2] ads.	A	##	##	+	—	—	—	—			
			B	##	+	—	—	—	—	—	—			B	+	+	###	###	##	+	—			
			O	—	—	—	—	—	—	—	—			O	###	###	##	+	—	—	—			
		Ads. by human A cells	A	—	—	—	—	—	—	—	—		Ads. by human A cells	A	—	—	—	—	—	—	—			
			B	+	±	—	—	—	—	—	—			B	+	###	###	##	+	—	—			
			O	—	—	—	—	—	—	—	—			O	—	—	—	—	—	—	—			
		Ads. by human B cells	A	###	##	+	—	—	—	—	—		Further[3] ads. by guinea pig cells	A	—	—	—	—	—	—	—			
			B	—	—	—	—	—	—	—	—			B	##	###	##	+	+	—	—			
			O	—	—	—	—	—	—	—	—			O	—	—	—	—	—	—	—			

TABLE 1. AGGLUTININ TITERS OF IMMUNE ANTI-BI, ANTI-BII AND ANTI-BIII SERA PREPARED FOR ADSORPTION TESTS (cont.)

Name of serum	Immune animal	Normal sera of imm. animal: Treatment	Human cell	Dilution 1	2	4	8	16	32	64	128	Immune antigen	Immune sera: Treatment	Human cell	Dilution 1	2	4	8	16	32	64	128	256	512
Anti-BIII no. 17 serum	No. 4 fowl male (o′)	No ads.	A	—	—	—	—	—	—	—	—	Guinea pig cell	No ads.	A	⋕	⋕	⋕	⋕	⋕	⋕	+	—	—	—
			B	—	—	—	—	—	—	—	—			B	⋕	⋕	⋕	⋕	⋕	⋕	⋕	⋕	+	—
			O	—	—	—	—	—	—	—	—			O	⋕	⋕	⋕	⋕	⋕	+	—	—	—	—
													Ads. by[3] human A cells	A	—	—	—	—	—	—	—	—	—	—
														B	⋕	⋕	⋕	⋕	⋕	⋕	⋕	⋕	+	—
														O	—	—	—	—	—	—	—	—	—	—
〃 〃 no. 20 serum	No. 8 fowl male (o′)	No ads.	A	—	—	—	—	—	—	—	—	〃	No ads.	A	⋕	⋕	⋕	⋕	⋕	⋕	+	—	—	—
			B	—	—	—	—	—	—	—	—			B	⋕	⋕	⋕	⋕	⋕	⋕	⋕	+	—	—
			O	—	—	—	—	—	—	—	—			O	⋕	⋕	⋕	⋕	⋕	+	—	—	—	—
													Ads. by[3] human A cells	A	—	—	—	—	—	—	—	—	—	—
														B	⋕	⋕	⋕	⋕	⋕	+	+	—	—	—
														O	—	—	—	—	—	—	—	—	—	—
〃 〃 no. 44 serum	No. 17 fowl female (♀)	No ads.	A	⋕	⋕	—	—	—	—	—	—	〃	No ads.	A	⋕	⋕	⋕	±	—	—	—	—		
			B	—	—	—	—	—	—	—	—			B	+	⋕	⋕	⋕	⋕	+	—	—		
			O	—	—	—	—	—	—	—	—			O	+	+	⋕	+	—	—	—	—		
		Ads. by human A cells	A	—	—	—	—	—	—	—	—		Ads. by[3] human A cells	A	—	—	—	—	—	—	—	—		
			B	—	—	—	—	—	—	—	—			B	+	⋕	⋕	⋕	⋕	+	—	—		
			O	—	—	—	—	—	—	—	—			O	—	—	—	—	—	—	—	—		

Remark: 1) Even before adsorption, no anti-B_{II} or anti-B_{III} antibody is not involved in this serum. (see Material 1))
2) This serum contains anti-B_{III} antibody besides anti-B_{II} one.
3) These sera are used for adsorption test with fin whale erythrocytes later on. 4) Serum type.

Adsorption tests of partial antibodies with fin whale erythrocytes. After purifying procedures each partial antibody was used for adsorption test with fin whale erythrocytes. Comparisons of their titers against

TABLE 2. ADSORPTION TESTS OF ANTI-BI, ANTI-BII AND ANTI-BIII AGGLUTININS WITH THE ERYTHROCYTES OF FIN WHALES

Name of serum	Treatment		Dilution of serum[3] 1	2	4	8	16	32	64	128	256	512	Remark
Anti-BI no. 13 serum	Control		++++	++++	++++	++++	++++	++++	++++	+	+	—	
	After ads. by	no. A29 Ju_1 cell	++++	++++	++++	++++	++++	++++	++++	+	+	—	No descent of titer
		no. A31 Ju_2 〃	++++	++++	++++	++++	++++	++++	++++	+	+	—	
		no. A45 Ju_1 Ju_2 〃	++++	++++	++++	++++	++++	++++	++++	+	+	—	
Anti-BII no. 45 serum	Control		++++	++++	++++	+	+	—	—	—	—	—	
	After ads. by	no. A29 Ju_1 cell	++++	++++	++++	+	+	—	—	—	—	—	No descent of titer
		no. A31 Ju_2 〃	++++	++++	++++	+	+	—	—	—	—	—	
		no. A45 Ju_1 Ju_2 〃	++++	++++	++++	+	+	—	—	—	—	—	
Anti-BIII no. 17 serum	Control		++++	++++	++++	++++	++++	++++	++++	++++	+	—	
	After ads. by[1]	no. 285 cell	++++	++++	++++	++++	++++	++++	+	+	—	—	Titer descend
		no. 286 〃	++++	++++	++++	++++	++++	++++	+	+	—	—	
		no. 287 〃	++++	++++	++++	++++	++++	++++	+	+	—	—	
		no. 288 〃	++++	++++	++++	++++	++++	+	+	—	—	—	
		no. 289 〃	++++	++++	++++	++++	++++	++++	+	+	—	—	
		no. 293 〃	++++	++++	++++	++++	++++	++++	+	+	—	—	
〃 〃 no. 20 serum	Control		++++	++++	++++	++++	++++	+	+	—	—	—	
	After ads. by[2]	no. 2 cell	++++	++++	+	+	—	—	—	—	—	—	Titer descend
		no. 3 〃	++++	++++	+	+	—	—	—	—	—	—	
		no. 4 〃	++++	++++	+	+	—	—	—	—	—	—	
〃 〃 no. 44 serum	Control		+	++++	++++	++++	++++	+	—	—	—	—	
	After ads. by	no. A29 Ju_1 cell	++++	++++	++++	++++	+	—	—	—	—	—	Titer descend
		no. A31 Ju_2 〃	++++	++++	++++	++++	+	—	—	—	—	—	
		no. A45 Ju_1 Ju_2 〃	++++	++++	++++	++++	+	—	—	—	—	—	

Remark: 1) and 2), Blood types of fin whale cells were not examined in these case.
3) Agglutinin titers against human B type erythrocytes.

human B cells between before and after adsorption are shown in table 2. Fin whale cells used for adsorption were examined previously on their blood groups (Ju-system only), but in cases of Nos. 17 and 20 sera these examinations were not made because of the lack of available reagent for blood grouping.

In this table agglutinin titers in the column of "control" show the pre-adsorbing titers against human B cells and the others show the the post-adsorbing those. As no descent of the titers is seen in the

anti-BI and anti-BII sera, it may be assumed that the corresponding B antigens, i. e. BI and BII, are not contained in the fin whale cells. While in the case of anti-BIII agglutinin descents of titers can be seen independently to blood groups of adsorbing cells at the degrees of 1/2 to 1/4 in no. 17 serum, 1/8 in no. 20 serum and 1/2 in no. 4 serum respectively. However, the anti-BIII agglutinin is not adsorbed thoroughly away in any case. From these facts it can be thought that the fin whale cells possess a portion of BIII antigen independently to their blood types, at least to Ju system. To confirm the existence of B antigen which seems to have simpler structure than BIII was worked out the following immunizing experiment.

Immunization of fowl with fin whale cells. In this experiment, prior to immunization the serum type and its titers of fowl's serum was examined, and after immunization the ascent of titer against human B cells was examined. As shown in table 3, such ascent of this titer was recognized at the degrees from 0 up to 8 times in no. 38 serum, from 0 up to 16 times in no. 47 serum and from 2 up to 16 times in no. 46 serum respectively. According to these results the existence of B partial antigen was immunologically confirmed.

All these anti-B agglutinins produced by the immunization was able to be completely adsorbed with both guinea pig cells and any other fin whale cells than immune antigens belonging to Ju1 type (nos. 47 and 48), Ju2 type (no. 59) and Ju1Ju2 type (nos. 45 and 48).

According to the results of adsorbing and immunizing tests in previous items 2) and 3), it was established that the B antigen in fin whale cells had the simpler structure than BIII in guinea pig cells. Though the comparative survey between this antigen and BIV in sheep and horse cells which was described by Ogura (1953b) has not been worked out, the author should like to use "BIV′" provisionally as the symbols of this substance. By using of this symbols the erythrocytes of B type human, rabbit, guinea pig and fin whale are shown to have following antigenic structures in relation to B antigen system.

B type human	:	BI BII BIII BIV′
rabbit	:	BII BIII BIV′
guinea pig	:	BIII BIV′
fin whale	:	BIV′

DISCUSSION

As already stated BIV′ antigen is contained in fin whale (*Balaenoptera physalus*) cells independently to Ju blood group system. And so far as author's survey reaches it can be assumed that this BIV′ partial antigen

TABLE 3. ANTI-B AGGLUTININS PRODUCED BY FOWLS IMMUNIZED WITH FIN WHALE ERYTHROCYTES

Serum No.	Fowl, immunized	Normal sera of fowl: Treatment	Cells for agglutinogen	Dilution 1	2	4	8	16	32	Immune antigen	Immune sera: Treatment	Cells for agglutinogen	Dilution 1	2	4	8	16	32	64	128	256	512	1024	2048	4096	Remarks
38	No. 16 male (α')[2]	No ads.	Human A	++++	+	—	—	—	—	No. 144 Ju_1 type cells	No ads.	Human A	++++	++++	++++	+	—	—	—	—	—	—	—	—	—	
			" B	—	—	—	—	—	—			" B	++++	++++	++++	++++	+	—	—	—	—	—	—	—	—	
			" O	—	—	—	—	—	—			" O	++++	++++	+	—	—	—	—	—	—	—	—	—	—	
			No. 144 fin Ju_1	—	—	—	—	—	—			No. 144 fin Ju_1	+	++++	++++	++++	++++	++++	++++	++++	++++	+	—	—	Titer ascend
											Ads. by human A cells	Human A	—	—	—	—	—									
												" B	++++	++++	++++	+	—									
												" O	—	—	—	—	—									
47	No. 20 female (α')	No ads.	Human A	++++	+	+	±	—	—	No. 40 Ju_1 type cells	No ads.	Human A	++++	++++	++++	++++	++++	++++	+	—	—	—	—			
			" B	+	—	—	—	—	—			" B	++++	++++	++++	++++	++++	+	—	—	—	—	—			
			" O	+	—	—	—	—	—			" O	++++	++++	++++	++++	+	+	—	—	—	—	—	—	—	
			No. 40 fin Ju_1	—	—	—	—	—	—			No. 40 fin Ju_1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	—	Titer ascend
		Ads. by human O cells	Human A	++++	+	—	—	—	—		Ads by human A cells	Human A	—	—	—	—	—									
			" B	—	—	—	—	—	—			" B	++++	++++	++++	++++	+	—								
			" O	—	—	—	—	—	—			" O	—	—	—	—	—									
46	No. 19 male ($\alpha'\beta'$)	No ads.	Human A	++++	++++	++++	+	±	—	No. 49 Ju_2 type cells	No. adsorp.	Human A	++++	++++	++++	++++	+	—	—	—	—	—	—			
			" B	++++	++++	+	—	—	—			" B	++++	++++	++++	++++	++++	++++	+	—	—	—	—			
			" O	+	±	—	—	—	—			" O	++++	++++	++++	++++	++++	+	—	—	—	—	—	—		
			No. 49 fin Ju_2	—	—	—	—	—	—			No. 49 fin Ju_2	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	—		Titer ascend
		Ads. by human O cells	Human A	++++	++++	+	—	—	—		Ads. by human A cells	Human A	—	—	—	—	—									
			" B[1]	++++	+	—	—	—	—			" B	++++	++++	++++	++++	+	—								
			" O	—	—	—	—	—	—			" O	—	—	—	—	—	—								

Remark: 1) This normal agglutinin was not adsorbed completely by human A type cells, but was adsorbed by guinea pig (B_{III}) cells. Therefore this agglutinin may be assumed to be one corresponding to B III or simpler structural B antigen than B III.

2) Serum type.

occurs in the cells of all fin whale individual independently to any other blood group systems of this species and constitutes a component of species-specific antigen of their erythrocytes. At present it has been immunochemically ascertained that the erythrocytes of sperm whale (*Physeter catodon*) and dolphin (*Prodelphinus caeruleoalbus*) possess some kind of more complicated structural B antigen than BIV', likely to be BIII (Fujino, unpublished data). In past days such analysis on B substance in whale cells has been reported by AZAMI (1949) alone. After investigating on two whales, he recognized the existence of BIII, still the specification on these whale species was not noted. However, such structural differences of B substance by whale species seem to offer to the author a prospect of discussion upon the evolutional relationship among various whale species, particularly between whalebone and toothed-whales. But any discussions on such problem should be taken up after obtaining the comprehensive data on the distribution of A, B and O blood group substances among cetaceans.

As previously cited, Ogura (1953b) reports that the erythrocytes of sheep and horse have a simpler structural B substance than BIII, and are to be named as BIV. Comparative survey between BIV and BIV' will be discussed in future issue.

NORMAL AGGLUTININ IN IMMUNE ANIMAL AND THEIR PRODUCING ABILITY OF IMMUNE AGGLUTININ

The author is working at immunogenetical researches on identification of breeding populations of whales in concerning to fishery problems (Fujino, 1956). To carrying out such extensive investigations it is very important to obtain immune antibodies, which have high specificities and high titers, for blood grouping.

After examining the normal agglutinins of animals specific to human A and B blood group antigens, Hibino (1935) introduces a term of " Serum type of animals ", and classifies the animal sera into four groups, i. e. $\alpha'\beta'$, α',β' and o', accordingly as the existence of anti-A or anti-B hetero-hemagglutinins. And then he illustrates that serum type of animal is a serological constitution, which is closely related to the producing ability of corresponding immune antibodies. While basing upon the quantity of human A-like substance in the saliva, Koshino (1938, 1939) divides rabbits serologically into two groups of secretor and nonsecretor, and confirmes that the nonsecretor is more excellent producer of anti-human A immune antibody than the secretor. Then he concludes that serum type and secretor-nonsecretor type of rabbit are important serological characters to be previously tested in case of preparation of immune anti-A antibody.

Same idea as abovestated points must be considered in preparation of the reagent for blood grouping on fin whales. The author notes here some data on the anti-Ju normal agglutinin in animal sera and the results of production of anti-Ju immune agglutinins by these animals. These data are not obtained under the intentional survey, but may serve to some extent for future work. As showing in table 4, the heterohemagglutinin specific to Ju1 antigen occurs in the normal serum of a fowl among three and the heterohemagglutinin specific to Ju2 antigen occurs in the normal sera of the three rabbits among five and a fowl among three. It can be seen from table 4 that the titers of anti-Ju2 immune agglutinins reach up to higher in rabbits (nos. 34, 35 and 36 sera) than in fowl (no. 46 serum), but in contrary to this tendency the titers of anti-Ju1 immune agglutinins reach up to higher in fowls (nos. 38 and 47 sera) than in rabbits (nos. 33 and 37 sera). In table 5 are shown some examples of agglutinin titers to explain the degree of specificity of these immune sera.

TABLE 4. AGGLUTININ TITERS OF NORMAL AND IMMUNE SERA PRODUCED BY RABBITS AND FOWLS IMMUNIZED WITH THE FIN WHALE ERYTHROCYTES

Immune animal				Normal agglutinin				After immuniz.		Immune[1] antigen		Serum No.
Species	No.	Sex	Serum type & A^+ or A^-	anti-Ju_1		anti-Ju_2				No.	Blood group	
				Exist.	Titer	Exist.	Titer	Anti-Ju_1	Anti-Ju_2			
Rabbit	33	male	$o'-A^+$	–	....	+	1: 8	1: 160	1: 8	111	Ju_1	33
〃	37	〃	$\alpha'-A^+$	–	....	–	....	1: 40	....	155	〃	37
Fowl	16	〃	α'	–	....	–	....	1: 800	....	144	〃	38
〃	20	female	α'	–	....	–	....	1: 640	....	40	〃	47
Rabbit	35	male	$\alpha'-A^+$	–	....	–	....	1: 20	1: 320	107	Ju_1Ju_2	35
〃	34	〃	$o'-A^+$	–	....	+	1: 64	....	1: 12800	113	Ju_2	34
〃	36	〃	$o'-A^-$	–	....	+	1: 64	....	1: 640	139	〃	36
Fowl	19	〃	$\alpha'\beta'$	+	1: 2	+	1: 4	1: 2	1: 160	49	〃	46

Remark: 1) Erythrocytes of fin whale.

According to this table it may be assumed that the degree of specificity of anti-Ju1 is rather low both in fowls and rabbits, and that the degree of anti-Ju2 specificity is low in fowl but rather high in rabbits, especially higher in those with normal anti-Ju2 agglitinin. After summarizing the abovestated points, followings seem to be concluded on preparation of anti-Ju1 and anti-Ju2 reagents.

1) To use rabbit that has corresponding normal agglutinin as the anti-Ju2 immune agglutinin producer.
2) To use fowl as the anti-Ju1 agglutinin producer.

By accomplishing the more extensive detection of normal anti-Ju1 agglutinin in fowl serum and of Ju blood group substances in various or-

TABLE 5. SOME EXAMPLES OF AGGLUTINATION REACTIONS OF ANTI-Ju1 AND ANTI-Ju2 IMMUNE (UNADSORBED) SERA AGAINST FIN WHALE ERYTHROCYTES

Serum no.	Cell antigen No.	Cell antigen Blood group	Dilution of antiserum 10	20	40	80	160	320	640	1280	2560	5120	10240	20480	40960	81920	Titers of blood group specific aggl.	Immune animal
38	111	Ju_1	##	##	##	##	##	##	#	+	—	—	—	—	—	—	Anti-Ju_1=800	fowl
	139	Ju_2	##	##	##	##	##	##	#	+	—	—	—	—	—	—		
33	159	Ju_1	##	##	##	##	#	#	+	—	—	—	—	—	—	—	Anti-Ju_1=160	rabbit
	158	Ju_2	#	##	##	##	#	#	+	—	—	—	—	—	—	—		
34	165	Ju_1	##	#	#	+	—	—	—	—	—	—	—	—	—	—	Anti-Ju_2=12,800	〃
	139	Ju_2	##	##	##	##	##	##	##	##	##	#	#	+	+	—		
35	168	Ju_1	##	##	##	#	+	—	—	—	—	—	—	—	—	—	Anti-Ju_1= 20	〃
	139	Ju_2	##	##	##	##	##	##	##	##	#	+	—	—	—	—	Anti-Ju_2=320	
46	40	Ju_1	##	##	##	##	##	##	#	#	+	—	—	—	—	—	Anti-Ju_2=160	fowl
	49	Ju_2	##	##	##	##	##	##	#	#	+	—	—	—	—	—		

gans or glands of immune animals, however, more excellent reagents must be obtained.

APPEARANCE OF IMMUNE ANTIBODIES INTO EGG-WHITE OF A FOWL INJECTED WITH FIN WHALE ERYTHROCYTES

No. 20 female fowl in table 3 (producer of no. 47 serum) was in a state of egg laying during the period of immunizing experiment. So the author

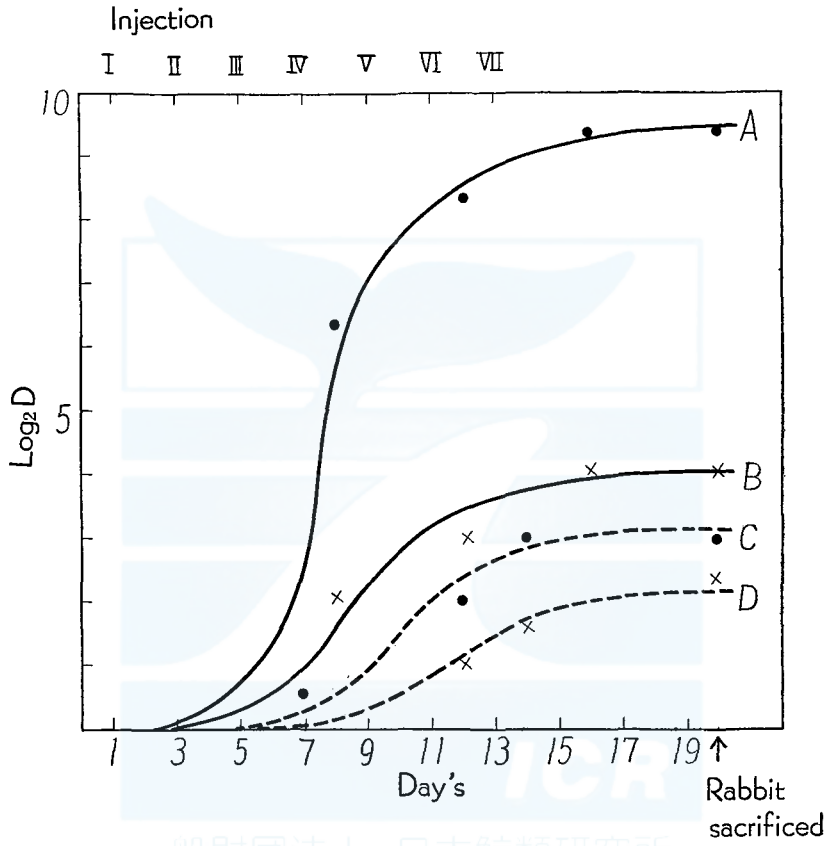

Fig. 1. Production of anti-Ju_1 and anti-B agglutinins in serum and egg-white of no. 20 fowl (D in $Log_2 D$=limiting dilution titers) A: anti-Ju_1 in serum, B: anti-B in serum C: anti-Ju_1 in egg white, D: anti-B in egg white.

tried to trace the appearance of immune antibody into egg-white. In the following are reported some notes on such observation. As already stated the no. 20 female fowl was given intravenously on alternate days a series of (seven-times') injections of 10% suspension of Jul type fin whale erythrocytes, and sacrified for bleeding 7 days after last injection. During the period of experiment this fowl laid a egg respectively in each 4th, 7th, 12th, 14th and 20th day from the start of injection, be-

ing up to 5 eggs in all. The author traced ascendings of anti-Ju1 and anti-human B agglutinin titers in these egg-whites and sera obtained by test bleedings. After centrifuging and inactivating, these agglutinins were titrated with hole-glass (slide glass) method. Limiting dilution titers of these agglutinins are plotted in figure 1.

It may be assumed from this figure that both anti-Ju1 and anti-B agglutinins in the egg-white begin to appear few days later than those in the serum and don't reach up to so high titers as those of the latter. As this observation was performed at the base of coastal whaling, no quantitative analysis have been carried out on globulins in egg-white and serum.

SUMMARY

1. Basing upon the classification of Friedenreich and With (1933) the author carried out the analysis of the human B blood group substance in the erythrocytes of cetaceans from the view points of systematic serology. A new partial antigen which has simpler structure than BIII in the guinea pig cells were detected immunologically in the fin whale erythrocytes independently to their blood groups. This antigen was named provisionally as BIV'. By this symbol the B blood group substances in the erythrocytes of human, rabbit, guinea pig and fin whale may be expressed as BIBIIBIIIBIV', BIIBIIIBIV', BIIIBIV', and BIV' respectively. Comparative survey between BIV' and BIV in the erythrocytes of sheep or horse (Ogura, 1953b) will be discussed in the future issue.

2. Normal agglutinins specific to Ju antigens of fin whale erythrocytes were detected in the sera of rabbits and fowls which were used as immune animal. In case of preparation of reagents for blood grouping on Ju system of fin whales, it is desirable that fowl is used as the immune animal for anti-Ju1 agglutinin producer and rabbit which has normal anti-Ju2 agglutinin is used as the anti-Ju2 agglitinin producer.

3. Appearance of immune antibodies into egg-white of a fowl was observed on the way of preparation of the anti-Ju1 agglutinin. It may be assumed from the results of this observation that both anti-Ju1 and anti-human B agglutinins begin to appear into egg-white few days later than into serum and don't reach up to so high titers as those of the latter.

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