THE JU BLOOD TYPING SYSTEM OF THE SPERM WHALE AND SPECIFIC SOLUBLE SUBSTANCES

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The use of immunogenetic concepts in marine population research is rapidly expanding (Cushing 1962) and pertinent material on whales has been reviewed (Fujino, 1960). In the case of sperm whales, a " species-specific " antigen resembling the human B antigen has been found on their erythrocytes, and antibodies for the human ABO system occur variably in their serums. Chicken and rabbit antiserums prepared against sperm whale erythrocytes have been used to distinguish six phenotypes among twenty-six individuals. One of the antigens designated, Sp, could also be recognized by a natural antibody in sei whale serum (Fujino 1954). The present paper reports additional work on sperm whale antigens that was done in California (Cushing and Calaprice), in the Antarctic (Fujino) and in Peru (Cushing).

CALIFORNIA RESEARCH

Samples of bloods from ten sperm whales taken by San Francisco shore whaling stations were preserved by glycerol-freezing (Cushing et al., 1959). Erythrocytes were recovered by dialysis and compared with those of finback, humpback, and sei whale erythrocytes with respect to their reactions with Fujino's (1956) heteroimmune anti-finback Ju1 (no. 47) and Ju2 (no. 34) serums. These comparisons demonstrated that, as in the humpback, pygmy-blue and sei whales, antigens occur in sperm whales that are very similar in specificity to the Ju1 and Ju2 finback antigens. These antigens behave in finbacks as if determined by a pair of allelic genes (Fujino, 1960).

The normal serums of horses, cattle, sheep and pigs were found to be useful reagents for detecting the Ju2 antigen. Antibodies for this antigen occurring in all serums examined. The serum of the California spiny lobster (*Panulirus interuptus*) was found to contain antibodies capable of agglutinating some Ju2-positive cells, but not others, suggesting that it may prove to be valuable as a Ju2 subtyping reagent (These reactions will be reported in a separate paper).

Isoantibodies in Ju2-negative sperm whales for Ju2-positive erythrocytes were found, as were cross-reactions between the Ju2-positive cells of one species with the isoantibodies of other species. These results suggest that the Sp antigen already referred to may have been Ju2 because of its agglutinability with sei whale serum. No other relations between the sperm whale antigens first described by Fujino and the ones described in this paper as like those of the finback Ju system can be determined, as the earlier anti-sperm whale reagents are no longer available

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for comparison.

The results given in the above section, together with relevant observations reported in earlier papers, establish the possibility of using the readily available reagents in normal serums for comparison of Ju2-positive reactions among sperm and other whale species in different parts of the world. The results of two such studies are given below.

ANTARCTIC RESEARCH

Data on the Ju system of blood types in Antarctic populations of sperm whales was obtained by Fujino while he served as biologist aboard the factory ship, Kyokuyo-maru No. 2 during the 1960/61 whaling season. Samples were taken from the heart of all but a few whales at an average time of eight hours post-mortem in sea and air temperatures which fluctuated within a few degrees of zero centigrade. The exceptional samples came from large clots of blood within the body cavity. Nine volumes of sample were added to one volume of the usual collecting solution. This consists of 8.5 gm. NaCl, 50 gm. of sodium citrate and 0.5 gm. of the antimicrobial

TABLE 1. ISOAGGLUTINATION OF VARIOUS WHALE ERYTHROCYTES BY UNDILUTED SERUMS*

Undiluted serums:		Ju2-positive cells													Ju2-negative cells	
		100	6	8	120	124	110	101	111	90	78	152	163	7	95	154
Ju2-neg. serum	95	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	++	+	±	−	−
	154	⧺	++	++	⧺	⧺	⧺	⧺	⧺	++	++	++	±	−	−	−
Ju2-pos. serum	163	++	++	++	++	++	++	++	−	−	−	−	−	−	−	−
	152	++	+	+	⧺	+	++	+	−	−	−	−	−	−	−	−
	101	+	+	⧺	⧺	−	−	−	−	−	−	−	−	−	−	−
	110	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	124	++	++	−	−	−	−	−	−	−	−	−	−	−	−	−
	111	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−
	120	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	100	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* See absorptions in Table 2.

These reactions show that subtypes exist among Ju2-positive cells and that the weaker subtypes can contain isoantibodies for stronger ones. Serums and cells of whales 95 and 154 are representative of those of all Ju 2 negative whales ⧺ symbolizes strong agglutination, ranging through ++ and + to − for no agglutination.

guanofuracin per liter of water. These samples remained in good condition for thirty days or longer under refrigeration. Serums of such samples showed little hemolysis, and had no opportunity to become mixed with other tissue fluids.

Each sample was tested with the same normal bovine serum, using conventional saline agglutination techniques. Agglutination was taken as evidence of the presence of Ju2 antigen. All Ju2 positive samples, together with various negative ones, were subsequently compared as to their reactions with normal horse serum, the same unabsorbed anti-finback serums used by Cushing and Calaprice, and the

TABLE 2. EXAMPLES OF ABSORPTIONS OF CATTLE SERUM (NO. 25) THAT SHOW SUBTYPE DIFFERENCES AMONG JU 2-POSITIVE SPERM WHALES*

	Cells:	Reactions with unabsorbed serum, dilutions: 1:2	1:4	1:8	1:16	1:32	1:64
Ju2 pos.	100	###	###	###	###	##	+
	124	###	###	###	###	##	+
	111	###	###	###	##	+	–
	7	###	##	+	–	–	–
Ju2 neg.	95	–	–	–	–	–	–

	Absorbing Cells:	Reactions with absorbed serums diluted one in eight, Test Cells 100	124	111	7	95
Ju2 pos.	100	–	–	–	–	–
	124	##	–	–	–	–
	111	###	###	–	–	–
	7	###	###	###	–	–
Ju2 neg.	95	###	###	###	+	–

* Also see Table 1 for reactions of these red cells with various whale normal serums.

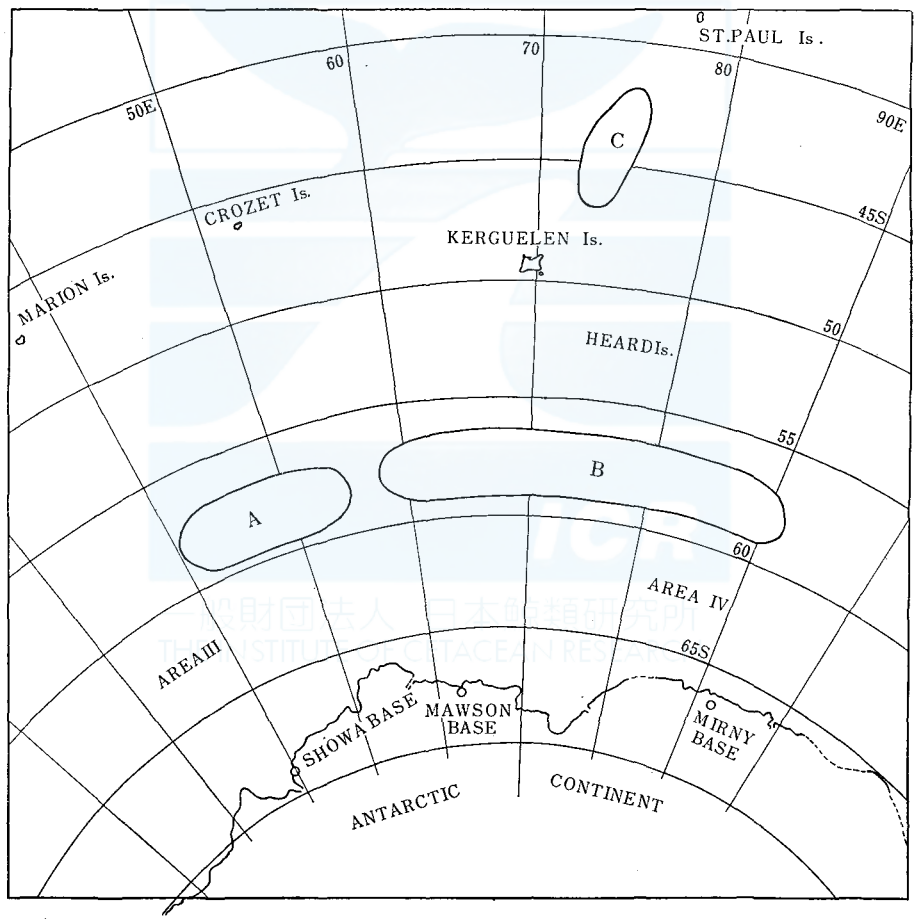

Fig. 1. Localities where sperm whales were taken by Kyokuyo-maru No. 2. in the 1960/61 whaling season.

isoantibodies in a single sperm whale serum. These comparisons continued to confirm the usefulness of normal animal serums as reagents for the detection of the Ju2 antigen on erythrocytes.

Isoantibodies for Ju2-positive cells were found in all Ju2-negative serums examined (one hundred and twenty-seven). In the weaker Ju2-positive subtypes were detected isoantibodies for the stronger Ju2 subtypes as showin in Table 1. Isoantibodies for Ju1-positive cells were not found.

All Ju2-positive samples from areas A and B (see Fig. 1), having been collected within a span of twenty-eight days, were compared with respect to their ability to absorb bovine serum. These absorptions showed that Ju2-positive cells exist as a series of at least four subtypes (Table 2). No subtypes of Ju2-negative cells were found following absorption of antifinback Ju1 reagent with cells from forty-six negative samples. Experiments were performed on the reactions of antibodies eluted from erythrocytes following absorption of bovine and anti-finback Ju1 serum. These will be reported in detail in connection with other related studies.

One whale was found that appeared to be a blood group chimera in that its cells consisted of a mixture of Ju2-positive and Ju2-negative cells as determined by microscopic examination of agglutionations, and by hemolytic tests.

TABLE 3. FREQUENCY OF OCCURRENCE OF BLOOD TYPES OF THE ANTARCTIC SPERM WHALES*

Blood types		Area A		Area B		Area C		Sum of all whales	
		Act. no.	Freq.	Act. no.	Freq.	Act. no.	Freq.	Act. no.	Freq.
Ju2-negatives		105	0.898	95	0.969	15	0.882	215	0.927
Ju2-positives, separated by subtypes.**	$Ju2_1$	1	0.102	1	0.031	1	0.118	3	0.073
	$Ju2_2$	2		0		0		2	
	$Ju2_3$	8		1		1		10	
	$Ju2_4$	1		1		0		2	
Sum		117	1.000	98	1.000	17	1.000	232	1.000

* See Fig. 1 for area limits

** Symbols of four subtypes of Ju2-positives were named in accordance with those in finbacks (Fujino, 1962).

The frequencies of Ju2-positive cells in population samples from the different Antarctic areas shown in Fig. 1 are given in Table 3. These are arranged accrding to subtype differences and suggest that larger samples will show that the sperm whale population of area A may differ from those of B and C.

PERUVIAN STUDIES

Sperm whale Ju blood types were studied in Peru by Cushing during July–August 1960 at the shore whaling station of Cia Balleneria Del Norte, Tierra Colorado

Bay, Paita, Peru. Whales were fished twenty to ninety miles off-shore and usually had been dead twelve to thirty hours before flensing. Blood samples were taken from various flensing cuts in the musculature, it being impractical to obtain heart blood as was done by Fujino. Whales in which decomposition was apparent were not sampled.

The erythrocytes obtained were in good condition, but the serum showed much hemolysis and was subject to mixt with fluids from the muscle tissue. A few milligrams of streptomycin and sulfathiazole were mixed with each 100 ml. sample, which kept well for several days refrigerated.

Conventional slide agglutination tests were used, being scored in a range between — for no reaction through ⧺ for the strongest agglutination. (Note that the score of agglutinations given by Fujino does not extend beyond ⧺. Study of the same materials by both authors while together in Tokyo showed this difference in convention had no effect on interpretations of the data obtained). Reagents used included the same anti-finback and stock of normal domestic animal serums referred to in the foregoing sections of this report. Emphasis on the Peruvian work centers on the reactions of erythrocytes with a pool of horse serum. As in previous work, saline controls were run on all cell suspensions to avoid possible error through autoagglutionation.

The horse serum readily distinguished two types of individuals. Cells reacting strongly were classified as Ju2-positive, those at dilutions of serum at one in five or less as Ju2-negative. Reactions with the anti-fin whale heteroimmune serums confirmed this classification.

As the first whale typed gave a strong positive reaction and the next three gave negative reactions, it looked as if the frequency of Ju2-positive cells would be readily obtained. However, a new phenomenon was encountered early in the investigation that showed this would be impossible to achieve within the time available. This phenomenon was the observation that five different samples which initially reacted as Ju 2-negative, changed type over a period of days and became Ju2-positive. In contrast, eight other Ju2-negative cells did not change type, nor did any Ju2-positive cells become negative.

As the changes occurred in spite of refrigeration and the presence of antibiotics, were Ju specific, and took place in samples in good condition, it seemed unlikely that they were due to microbial actions (Stormont et al., 1960).

The possibility that the erythrocytes had acquired a specific soluble Ju2 substance (hereafter Ju2 SSS) from the serum or tissues was therefore investigated. Such substances were first described by Stormont who showed that the J types of cattle erythrocytes are determined by SSS in the blood plasma (Stormont, 1949). They have subsequently been found in man, sheep and other domestic species (An introduction to recent literature on soluble blood group antigens will be found in Sneath et al., 1959, Stone, 1962, Stormont et al, 1960, and Race et al., 1958). Limited observations on sperm whale human B-like antigen and on the finback Ju system have already suggested their occurrence in cetaceans (Cushing et al., 1959, Fujino, 1960).

Table 4 shows the reactions of the whales that changed type. While circumstances did not permit an extensive series of experiments, several kinds of observations were made which indicate that the type of change was actually due to postmortem coating of erythrocytes with Ju2 SSS.

The first of these observations was the demonstration that Ju2 SSS capable of inhibiting the reactions of horse serum with Ju2-positive cells occurs in the serums of whales that changed types, and in examples of other positive whales (Table 5). The failure of inhibition with respect to certain combinations of cells and serums can be reasonably explained as due to subtype differences among the reactants involved. Serums of four whales typed as Ju2-negative did not inhibit the action

TABLE 4. REACTIONS SHOWING CHANGE IN JU TYPE OF THE ERYTHROCYTES OF INDIVIDUAL WHALES

Whale nos:	Horse serum dilutions 1 : 5	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	Date tested, 1960
3	–	–	–	–	–	–	7–31
3	###	###	###	##	+	–	8–2
*3	–	–	–	–	–	–	8–18
19	+	–	–	–	–	–	8–3
19	###	###	###	###	###	##	8–5
19	###	###	###	###	###	+	8–15
21	–	–	–	–	–	–	8–3
21	–	–	–	–	–	–	8–5
21	##	##	##	+	–	–	8–7
21	###	###	###	###	##	+	8–15
5–5	+	–	–	–	–	–	8–6
5–5	##	##	+	±	–	–	8–7
5–5	###	##	##	##	+	–	8–9
5–5	###	###	###	###	##	–	8–13
4–3	–	–	–	–	–	–	8–5
4–3	###	###	###	###	+	–	8–9

* This sample represents cells recovered from an aliquot of 3 blood frozen in glycerol citrate and recovered by dialysis Aug. 18. The cells had not become positive during this time, in contrast to the change in unfrozen cells.

of horse serum. Account was taken of the occurrence and action of isoantibodies in these and other serums where these antibodies might have complicated results.

Another kind of supporting evidence are the results of some absorptions of horse serum involving the erythrocytes of some of the whales that changed types, and which are shown in Table 5 to have Ju2 SSS in their serums (Table 6). These absorptions show that, as in the inhibition tests, individual variations exist in the reactions of different erythrocytes with different serums. The relations between 19 and 21 are strikingly similar in both kinds of experiments. Cells 4–1 and 5–6 removed antibodies agglutinating some other kinds of cells while not agglutinating to any degree in unabsorbed horse serum themselves ; also that the serums of these cells were capable of inhibition (Table 5). This is a situation similar to that reported for certain of the cattle J types (Stormont et al., 1960, Stormont, 1962).

A third kind of experiment further supports the possibility that the type changes

TABLE 5. EXAMPLES OF THE INHIBITION OF HORSE SERUM BY SERUMS FROM JU2-POSITIVE WHALES. THOSE THAT CHANGED TYPE ARE INDICATED BY AN ASTERISK

Test cells	horse serum only	*21	*4-3	*5-5	*0-3	0-1	5-6	4-1	*19
5-5	++++	—	+	+	+	+	++	++	++++
4-3	++++	—	+	+	+	+	++	++	++++
21	++++	—	++	++++	++++	++++	++++	++++	++++
0-1	++++	++++	++++	++++	+	—	++	++	+
19	++++	++++	++++	++++	+	—	++	++	++

Horse serum diluted one in ten plus individual whale serums (excepting first column which shows the reactions of horse serum alone). One drop of whale serum was added to one drop of horse serum for each test. Cells were added fifteen minutes later and readings made after an additional twenty minutes. ++ indicates strong agglutination and therefore no inhibition, while —indicates no agglutination and therefore complete inhibition. Note that homologous combinations 4–3, 5–5 and 19 did not show complete inhibition. No isoagglutinins for the cells used in the above tests were found in serums 21, 5–5, 0–1, 5–6, and 19. Serums 0–3, 4–3, and 4–5 were not tested for isoagglutinins. Four Ju2-negative serums (0–2, 0–4, 4–5, and 11–7) were found to contain isoagglutinins for the above cells, and did not inhibit the agglutination of these cells by horse serum.

TABLE 6. THE REACTIONS BETWEEN VARIOUS ABSORBED HORSE SERUMS AND ERYTHROCYTES

	Absorbing cells	Test cells 5-6	4-5	4-1	19	21	4-3	4-4	8-3	4-2	5-5	6-2
Unabsorbed serum (1 : 10)	none	—	—	+	++++	++++	++++	++++	++++	++++	++++	++++
Absorbed serums (1 : 10)	19 Ju2-pos.	—	—	±	+	++++	++++	++++	++++	++++	++++	+
	21 Ju2-pos.	—	—	—	++++	—	—	++	++	++	++	++
	4-1 Ju2-neg.	—	—	—	++	++	+					
	5-6 Ju2-neg.	—	—	—	++++	++++	++					
	4-5 Ju2-neg.	—	—	—	++++	++++	++++					

Blank spaces in columns to right show that no tests were made. Serums were absorbed at room temperature with one-third volume of packed cells for thirty minutes. Results show the marked reciprocal relationship between 19 and 21 observed in inhibitions (table 5). Note that absorption with cells of 19 was not complete. Other heterogeneities are revealed suggestive of the subtypes found by Fujino. Note that cells 4–1 and 5–6, while reacting essentially negatively with horse serum, were still capable of removing some of the antibodies or other erythrocytes reacting with this serum.

observed were due to the coating of erythrocytes with Ju2 SSS. One experiment of this kind was performed by placing drops of washed erythrocyte suspensions from two Ju2-negative whales (10–2 and 11–7) in drops of undiluted serum from a Ju2-positive whale (11–11). These mixtures were allowed to stand for twenty minutes, at the end of time which ± traces of agglutination attributed to agglutinins other than those involved with the Ju system were observed. No agglutination occurred in saline controls of the cells or in the mixture of 11–11 cells with their homologous serum.

Drops of horse serum (1 : 5) were then added to the three mixtures of cells with 11–11 serum. At the end of a second period of twenty minutes cells of 10–2 and 11–7 showed strong (卌) agglutination while those of 11–11 were negative. In contrast horse serum placed in the saline controls gave only a ± agglutination with 10–2 cells, none with 11–7 cells, and 卌 with 11–11 cells. These results can be interpreted as due to the coating of the erythrocytes with Ju2 SSS in the case of cells 10–2 and 11–7, and to the inhibition of horse serum in the case of 11–11. They were confirmed in a second series of experiments that showed the titer of Ju2 SSS inhibitor in 11–11 serum to be one in sixteen with respect to the reactions of horse serum with 11–11 cells ; and one in four with respect to ability to coat the cells of 11–7. (The serum of 11–7 contained strong iso-antibodies for 11–11 cells and for all the Ju2-positive cells shown in table 5. It did not inhibit the reactions of horse serum with these cells).

A second experiment similar in kind to the one just described was performed by placing a suspension of human type A cells in the serum of anotner Ju2-positive whale 5–3. This serum was also known to inhibit the agglutination of its homologous cells by horse serum. No agglutination of the human cells occurred in this serum after twenty minutes. The adition of horse serum (one in ten) to the mixture again resulted in a strong agglutination after a twenty minutes wait. The cells of Ju2-negative whale 0–2 also were affected by 5–3 serum in the same way. Controls showed that the horse serum alone gave only traces of agglutination with the human cells and none with the 5–3 whale cells.

A third experiment was made with the serum of Ju2-positive whale 0–1 also known to contain horse serum inhibitor and not to contain isoantibodies. In this case, however, it was not possible to induce the agglutination of human A cells or those of Ju2-negative whale 0–2. The serum of whale 0–2 did not appear to contain Ju2 SSS as shown by an inhibition experiment which took into account the presence of isoagglutinins in the 0–2 serum.

One set of experiments was made which showed that the serum of Ju2 positive whale 11–11 strongly inhibited the agglutination of its own cells by finback anti-Ju2 serum (no. 34) as well as that of the cells of Ju2 positive whale 0–1.

DISCUSSION

While alternative explanations can not yet be ruled out, the observations reported above support the concept that the changes in type of sperm whale erythrocytes were the result of post-mortem coating with Ju2 SSS. Changes of a similar nature have been produced experimentally with the cattle J antigen, originally reported by Stormont (1949) and more recently by Stone (1962). It is also of some interest that Stone et al (1954 p. 399) comment that occassionally a sample of cattle cells classified as J negative later showed very weak reactions with high concentrations of J reagents, and that these individuals occurred among those whose serum contained J inhibitor. Experiments reported by Kodani (1962) show that the blood group phenotypes of human epithelial cells can also be altered by exposure to

soluble heterologous blood group substances. The occurrence of Ju2-negative whales whose serum contained isoantibodies for Ju2-positive cells, and which were not capable of inhibiting the action of horse serum, show that the two major classes of whales found in California and the Antarctic also exist in the Peruvian population. However, the frequency of individuals whose erythrocytes reacted strongly with horse serum (including the five that changed type) was much higher in the Peruvian sample than in the others. Such whales totaled 25 among a sample of 33 whales that were systematically typed with horse serum and the two antifinback heteroimmune serums. (Whales 4–1 and 5–6 are counted as negative in this instance as they did not agglutinate in horse serum).

The tentative explanation offered for the high frequency of Ju2-positive erythrocytes in the Peruvian material is based on the fact that the blood was obtained from whales in a relative advanced post-mortem condition due to a longer period, in higher temperatures of sea and air, between death and flensing. In addition, the samples, having been taken from supercial cuts rather than the heart, were exposed to mixture with body fluids other than serum. These factors could well provide a reasonable opportunity for Ju2 SSS to coat the erythrocytes after the death of the animal. If this explanation is correct the Ju2 antigen, by comparison with Antarctic data, would seem to occur on the erythrocytes of living individuals with much less frequency than it occurs in the serum and or other tissues as a soluble substance.

The above considerations obviously lead to an evaluation of the usefulness of the Ju antigens in population reaserch. Two points are to be made. First, the phenomenon of type change was not observed by Fujino in his studies on sperm whales. This supports the view that fresh blood samples taken from the heart present an accurate picture of the relations between Ju2 antigen in the serum and attached to erythrocytes. Second, no type changes have been observed in the very extensive research that has been done on finback whales. Here not only are the conditions of sampling significant, but also the fact that this species, being a baleen whale, is very far removed in an evolutionary sense from the sperm whale. This is significant as it is known that the human ABO substances vary with respect to their solubility and occurrence upon erythrocytes among the many species where they occur (see, for example, Mourant 1954 page 175 on the primates). Other points could be made, but enough has been written upon which to base two conclusions from the results of this paper. First, the Ju2 finback-like antigens in sperm whales appear to be soluble antigens with properties akin to the soluble antigens known in other species. Second, samples taken from the heart blood of recently killed whales appear to present valid material for frequency determinations. However, further blood typing studies involving the Ju system will have to consider the possible complexities introduced by soluble components of this system and methods of sampling. That the frequency of the different phenotypes produced by systems involving soluble antigens can be determined is already shown in them literature on the occurrence of cattle J in various breeds (Sprague 1958, Stormont 1959) and by the studies that have been made on the human Lewis system (Race

et al. 1958).

SUMMARY

This paper reports the discovery of a pair of antigens in sperm whales that resemble the Ju1 and Ju2 antigens of finbacks and other species. Reagents for the detection of these antigens include heteroimmune antifinback serums, the normal serums of several domestic animals, and isoantibodies for Ju2 in sperm whale serum. The Ju2 antigen exists as a series of at least four subtypes. The distribution of Ju2-positive individuals was determined in Antarctic samples of sperm whale populations. Observations on sperm whales taken off Peru suggest that the Ju2 antigen can exist as a soluble substance which can cause changes of type in erythrocytes on post-mortem.

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