Blood Groups in the Atlantic Bottle-Nosed Porpoise (*Tursiops truncatus*)[1] (35588)

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(Introduced by S. I. Rapaport)

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The study of Ridgway and McCormick (1) showed that it is possible to anesthetize porpoises for major surgery. During studies in the training of porpoises, it was deemed necessary to do ear surgery—and, in one case, a hysterectomy—on some of the animals. Considerable blood loss was associated with these operations. It was apparent that it would be best if compatible blood were available for transfusion during these operations, and to provide this compatibility, there must be some knowledge of the blood groups. In the current literature, considerable interest has been evidenced in blood groups of the various domestic and wild animals (2), and a relatively recent review of the blood groups in marine animals has been published (3). Cushing *et al.* (4) have demonstrated the presence of two blood group alleles (Ju 1 and Ju 2) in sperm whales, but there appears to be no studies on the presence of such blood groups in other Cetacea. This report presents a study showing the presence of naturally-occurring saline reactive blood group agglutinins in porpoise serum; which demonstrate blood groups in these animals.

Materials. Sera. Blood was drawn from a central vessel of the tail fluke and allowed to clot. Clotting takes place in 30–60 min at room temperature. This prolonged clotting time is due to a deficiency of Hageman factor (factor XII) which has been reported by Robinson *et al.* (5). If it became necessary to accelerate the clotting time, a small amount of bovine thrombin was added. After clotting, the blood was centrifuged, and the serum was separated, frozen, and stored at −20°.

Red blood cells. Nine ml of freshly shed blood was placed in a tube containing 9 mg of disodium ethylenediaminetetraacetic acid (EDTA). The red cells were washed in 0.9% NaCl solution, and reconstituted to a 4% suspension in saline prior to use. To preserve the cells for long periods, they were mixed with a 0.45 *M* glucose–sucrose solution and sprayed into liquid N_2. Thawing was carried out by adding the frozen cells directly to 37° saline.

Rabbit antiporpoise gamma globulin. Sera from 4 porpoises were pooled and the globulins were precipitated with ammonium sulfate. The precipitate was washed, mixed with potassium aluminum sulfate (potassium alum) and injected intramuscularly into rabbits (6). At weekly intervals the rabbits were bled and their sera were separated. The sera were serially diluted, and the dilutions were reacted with latex particles coated with porpoise gamma globulin. The highest serum dilution producing agglutination of the particles was recorded. The rabbits were repeatedly immunized until an agglutinating titer of at least 32 was obtained. The rabbits were then exsanguinated and their sera were separated and frozen at −20°. Before use the sera were absorbed with saline-washed porpoise cells until no hemagglutinating activity was present.

Methods. Initial studies showed that when the animal sera were mixed with their own red cells and then cooled to 4°, autoagglu-

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tination occurred in about 75% of the tests. Autoagglutination was usually not present if the reaction was carried out at 12°. To remove the autoagglutinin, the sera were repeatedly absorbed overnight at 4° with their own packed, saline-washed red cells, until no subsequent autoagglutination occurred.

The remainder of the studies were carried out using porpoise cells and autoabsorbed porpoise sera. Equal volumes of a porpoise serum and a 4% suspension of red cells in saline were combined in a test tube. This mixture was treated by various methods. First, they were incubated either at 4, 10, 16, or 37°; centrifuged, and agglutination was determined. In a second study, the red cells were pretreated with a ficin solution, incubated with serum at 16° for 0.5 hr and agglutination determined. Thirdly, bovine albumin (22%) was added in equal volume to the mixture of cells and serum. The resulting combination was incubated at 37° for 1 hr and the cells were centrifuged. Last, the cells and serum were incubated for 1 hr at 37°; the cells were washed thrice with saline, after which rabbit antiporpoise gamma globulin serum was added and the cells were centrifuged. It was found that serological specificity was demonstrated with a crisp reaction by incubation of the cell–serum mixture at 16° and was not enhanced by the use of any other method. All further studies were performed with this method.

When the red cells and serum were mixed and reacted, it appeared the red cell reactions could be arranged in several major groups, but considerable cross reactivity could be found. To clarify the reactions, the sera were absorbed with the weakly reactive red cells to remove trace reactions. Eluates were prepared from the absorbing red cells using heat elution at 56°; the eluate was then tested to see if the absorbed antibodies had significant red cell activity.

Blood was drawn simultaneously from 5 animals and the red cells and sera cross reacted. The results are shown in Table I.

It may be seen that one animal (Stormy) had red cells which did not react with any of the sera studied. Additionally, her cells, as well as those of several other animals subsequently tested did not react with 38 other sera. One animal (Gilli) had cells which reacted with two sera, (Type 1), while three animals (Wave, Ops, and Skipper) had cells which reacted with two other sera (Type 2). The serum reactions are interpreted that Stormy with Type O cells had anti-2 in her serum; as did Gilli, whose red cells were Type 1. Ops and Skipper, whose red cells were Type 2, had anti-1 in their serum; while Wave, whose red cells were of the same type, had no antibody present in her serum.

TABLE I. Crossmatch Studies Between Red Cells and Sera of Five Porpoises.

Red cell reactions are in vertical columns, antisera in horizontal columns.

	Red cells				
	Stormy	Gilli	Wave	Ops.	Skipper
Sera					
Stormy	—	—	+	+	+
Gilli	—	—	+	+	+
Wave	—	—	—	—	—
Ops.	—	+	—	—	—
Skipper	—	+	—	—	—

To date, 39 animals have been studied and four have been found which fit the category of Type O. The cell grouping patterns of the other animals previously tested are not as clear as is this last group of 5, due to the presence of weak antibodies or aberrant reactions. These bloods are being studied further to see if repeated absorption will clarify the results. No genetic or family studies have been performed, since no breeding pairs are available, and since the animals breed very poorly in captivity.

To determine if *in vitro* incompatible cells would survive; autologous, blood group compatible, and blood group incompatible red cells were labeled with ^{51}Cr and infused into recipients. The survival half-life of the autologous cells was 16.5 days, and the compatible cells was 13.5 days, while the half-life of the incompatible cells was 7.4 days (7). It, therefore, appears that intraspecies *in vitro* incompatibility signifies a short *in vivo* half-life.

During the course of this work, it became necessary to transfuse 4 different animals due

to blood loss during surgery. Compatibility was determined by crossmatching using the red cells in saline suspension, as previously outlined. In each case, 500 ml of blood were drawn from a Type O donor into a sterile bottle containing NIH-ACD solution B and the transfusion was then performed with no apparent change in the animal's vital signs. No hematuria was observed, nor was there any evidence of hemoglobinemia.

Discussion. Although the blood groups of various animals have been studied in detail, the porpoise had attracted little attention. Much of this lack of interest is probably due to the unavailability of large numbers of these animals in captivity and also to the almost complete lack of captive breeding pairs. The presence of naturally-occurring saline agglutinins is not unexpected. The symposium edited by Cohen (2) lists a number of animals which have naturally-occurring agglutinins, and others which do not. There appears to be no clearly defined evolutionary classification for the presence of these antibodies. We assume these antibodies are naturally-occurring since the animals have not been immunized with blood. On the other hand, all the animals are injected on capture with swine erysipelas vaccine; and it is conceivable that this immunization could stimulate antibody production if the antigens of the vaccine and the red cells cross react. No studies have been performed to test this hypothesis.

The definition of the naturally-occurring agglutinins was made more difficult by the added presence of cold reacting auto agglutinins, but these could be removed by repeated absorption with autologous red cells. It is difficult to see the need for these cold agglutinins. The porpoise's life is spent conserving his body heat from a colder exterior environment. In this case, it would seem that cold agglutinins would be a decided handicap, since sooner or later, especially in the flukes, his blood would encounter cooler temperatures, but apparently the phenomenon is not severe enough to produce symptoms *in vivo*, if indeed there is an *in vivo* reaction at all. Certainly no evidence of *in vivo* reactvity was noted when the bloods were transfused, so we assume this reaction is only an *in vitro* curiosity.

It would be much more tidy if the Group O blood had both anti-1 and 2, rather than just anti-2. Similarly, all three Group 2 bloods should have anti-1, whereas actually only two have it. This phenomenon could be explained by a chance absence or weak reactivity of anti-1, or, more likely, that a weak anti-1 was removed during the absorption with homologous cells. The eluate from these cells did not show any anti-1 activity, but this antibody would not have been demonstrable in the eluate if it was not very strong before absorption. Studies on more animals will clarify this finding.

The choice of a numerical nomenclature for the blood group was done with full knowledge of how confusing a numerical nomenclature can be. Since no family studies have been done, it would be folly to apply some type of classification implying a genetic basis. It is hoped the numerical nomenclature will be replaced in the near future with a correct genetic terminology.

Other than the animals transfused, no animals were deliberately immunized during this study. If this had been done, probably more antibodies would have been found. In addition, it is expected that as the population of studied animals is enlarged, more blood groups will be found, and hopefully the genetics clarified.

Summary. A study of porpoise blood has shown the presence of "naturally-occurring" agglutinins in the serum which demonstrate three blood groups. These blood groups influence the survival of transfused cells, and thus have clinical significance. Before transfusion, donor bloods must be cross-matched with the recipient, even when the recipient has had no prior transfusions.

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