BLOOD TYPING OF DRIED WHALE ERYTHROCYTES WITH ^{131}I LABELLED ANTIBODY*

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Blood typing techniques and concepts are now being applied in population studies of several species of marine vertebrates important in fisheries (Fujino, 1956; Suzuki, Shimizu & Morio, 1958; Ridgway, Cushing & Durall, 1958). A major problem of this work is the preservation of samples of blood until they can be brought into a laboratory for analysis. One method of preservation that has been found to be useful is that of glycerol-freezing (Cushing, Fujino & Takahashi, 1959). The present paper is a continuation of research initiated by the senior author (Fujino, 1958) on the possibility of developing another method, using dried blood and radioactive antibody. The usefulness of ^{131}I labelled antibody for this purpose was suggested by a variety of researches (for example, Pressman & Eisen, 1950; Bournsnell, Coombs & Rizk, 1953).

MATERIAL AND METHOD

Blood samples from blue-white dolphins (*Stenella caeruleo-albus*) were taken from individuals captured at Ito, Shizuoka Prefecture. Dried samples were prepared by diluting one part of fresh whole blood with nine parts of 1.5% NaCl solution, being careful to equilibrate the colorimetric concentration of different samples. Single drops of diluted blood were spotted on pieces of filter paper (Toyo filter paper No. A.5) and allowed to dry at room temperatures. These samples were then preserved in a dessicator until they were used, approximately three months after collection. A second set of dolphin bloods was preserved by glycerol-freezing as described in the paper on this subject cited above (Cushing, Fujino & Takahashi, 1959).

The antiserum used was prepared by injecting a rabbit with fresh dolphin cells from a single individual (S. 23). Absorption of the antiserum obtained with heterologous dolphin cells (S. 25) left antibodies specific to the homologous cells as shown in Table 1. (The antigen

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TABLE 1. AGGLUTININ TITER OF ANTI-23 DOLPHIN SERUM IN EACH STAGE OF TREATMENT

Treatment of antibody	Before or after absorb. by S. 25. dolphin cells	Dolphin cells used for agglutination	Dilution of antibody							
			8	16	32	64	128	256	512	1024
Before salting-out	before	S. 23	++++	+++	+++	+++	+++	++	+	−
		S. 25	+++	+++	+++	++	+	−	−	−
	after	S. 23	+++	++	+	+	−	−	−	−
		S. 25	−	−	−	−	−	−	−	−
After salting-out	before	S. 23	+++	+++	+++	+++	++	+	−	−
		S. 25	+++	+++	+++	++	+	−	−	−
	after	S. 23	+++	++	+	±	−	−	−	−
		S. 25	−	−	−	−	−	−	−	−
After iodination	before	S. 23	+++	+++	+++	++	++	+	−	−
		S. 25	+++	+++	++	+	−	−	−	−
	after	S. 23	+++	++	+	±	−	−	−	−
		S. 25	−	−	−	−	−	−	−	−

involved has not yet been related to the Dc system previously described for this species by Yamaguchi & Fujino, 1952).

Iodination procedures followed those described by Pressman & Eisen (1950). Antibody gamma-globulin was precipitated from the immune serum following one third saturation with ammonium sulfate at ph 7.0. The ammonium sulfate was removed from the gamma-globulin fraction by a series of dialysis against isotonic saline, and against carbowax of molecular weight 1,500 (Arai, Sakagishi & Nomiyama, 1956). The solution was restored to its original volume following dialysis. The success of dialysis was checked with barium chloride indicator.

The protein concentration of the final preparation was determined chemically by the semi-micro Kjeldahl method, and colorimetrically with the Beckman spectrophotometer at wave length 545 $m\mu$ using Sols' Biuret reagent (Sols, 1947; Matsumoto & Kanamitsu, 1955). A conversion factor was calculated as follows: $P=1.16 \cdot D$, where P is the concentration of protein nitrogen in *mg.* per *ml.* and D ($=ln\, I_0/I$) is the optical density per 1 *cm.* depth of solution at wave length 545 $m\mu$.

Radioactive iodine ^{131}I, manufactured by the Radiochemical Centre, Amersham, England, was used for labelling the antibody. This is prepared as the sodium iodide in 0.01 *N* sodium thiosulfate. Iodination was accomplished by adding the iodinating solution (0.1 *ml.* of 0.01 *M* potassium iodide, 1.0 *ml.* of carrier free ^{131}I at 0.1 *mc.*, 1 drop of 1 *M* sodium nitrite and 0.2 *ml.* of 2.5 *N* hydrochloric acid) to a solution containing 15.0 *mg.* of gamma-globulin in 2 *ml.* of borate buffer at ph 8.0 (0.16 *N* sodium hydroxide, 0.2 *M* boric acid in 0.16 *M* sodium chloride). After five minutes the ph of this solution was adjusted to 8.0 with 2.5 *N* sodium hydroxide. Twenty minutes later dialysis was started against saline and carbowax as before.

After dialysing, completion of removal of the uncoupled ^{131}I was checked by the radioautography from paperchromatograph of iodinated antibody as well as by counting of radioactivity of dialysates with G-M counter. Fig. 1 shows an example of radioautographs made of an anti-finback whale serum that demonstrate the method used. These show

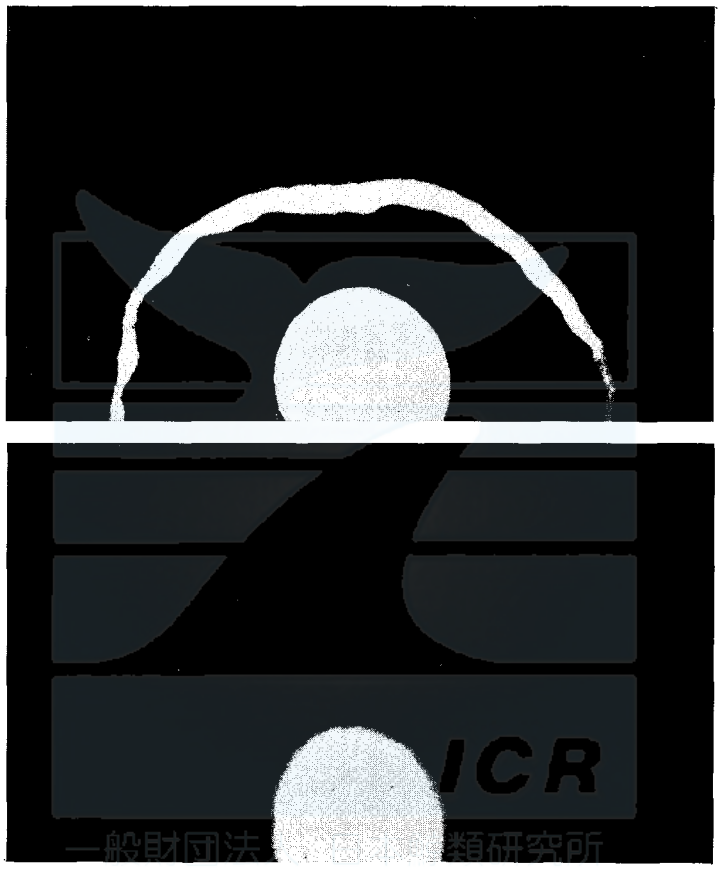

Fig. 1. Radioautographs from paperchromatograms of iodinated antibody, showing the complete removal of uncoupled ^{131}I from iodinated anti-finback antibody, Toyo filter paper 5A, 50 minutes diffusion in solvent which consists of 1 part of acetic acid, 3 parts of normal butyl alcohol and 4 parts of distilled water, Fuji-X-ray film, 6 hrs. exposed, upper: just after iodination, lower: after removal of uncoupled ^{131}I by dialysis. The central spot in each autograph shows the location of iodinated anti-body-gamma-globulin. The band in the upper figure shows the location of migrating uncoupled ^{131}I. This band is absent in the lower figure after a comparable time of diffusion.

that the serum proteins have been successfully iodinated and that the free ^{131}I was removed successfully by dialysis.

TABLE 2. RESULTS OF COUNTING TOTAL RADIOACTIVITY OF BLOOD SPOTS ON FILTER PAPER

Experiment	Dolphin cells	Count of radioactivity in c.p.m.[1)]
Preliminary test with intact cells	S. 23	1811±294
	S. 25	51± 31
Test with dried materials	S. 23	4077±748
	S. 25	221± 76

1) Mean values and standard deviations of five separate experiments. Cells were treated at a dilution 1:8 with iodinated antibody previously absorbed with S. 2.5 cells.

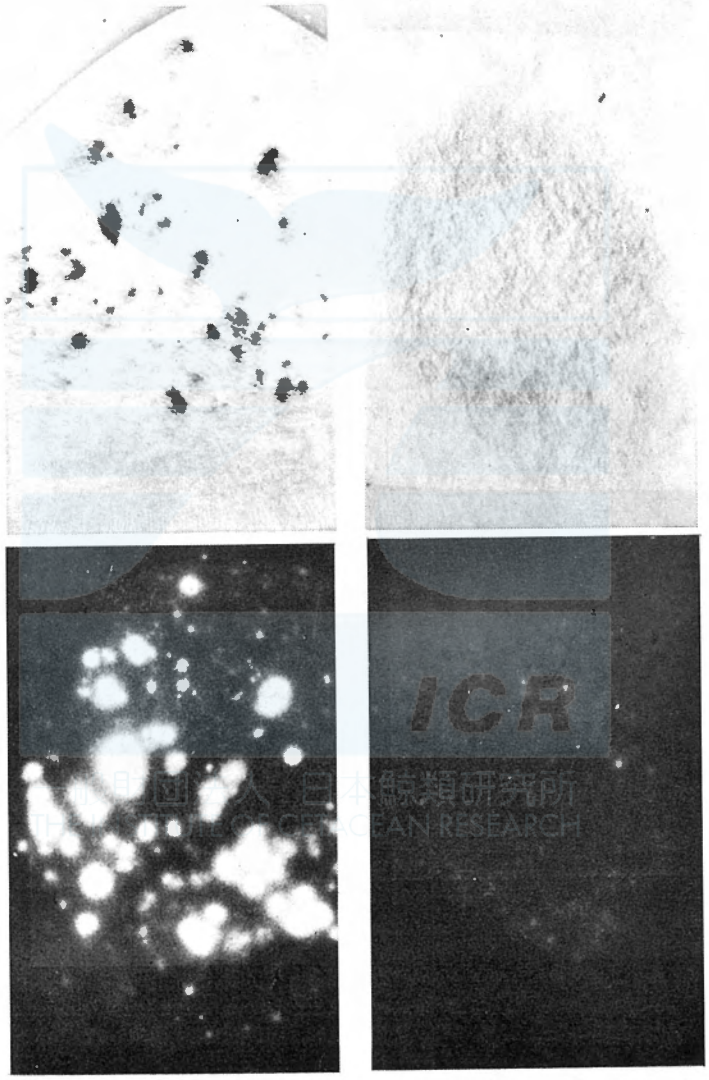

Fig. 2. Spots of dolphin cells on filter paper and their radioautographs after treatment by ^{131}I-labelled antibody, Fuji-X-ray film, 100 hrs. exposed, left: positive antigen-antibody reaction of S. 23 cells, right: negative antigen-antibody reaction of S. 25 cells.

BLOOD TYPING PROCEDURE

After titration the labelled antiserum was absorbed three times with the cells of dolphin S-25 which removed antibodies capable of reacting with antigens common to both individuals, as well as the remaining traces of uncoupled ^{131}I (Table 1).

The reactions of the absorbed antiserum with intact erythrocytes were observed first. Three drops of a two percent cell suspension from dolphin S-23 were added to a test tube containing three drops of one in eight dilution of the absorbed antiserum. A similar mixture was made with S-25 cells as a control. After thirty minutes each of the cell suspensions was centrifuged and was washed twelve times with saline to remove uncombined antibody. The whole washed cells were spotted on filter paper, dried at room temperatures, and their total radioactivity was determined by G-M counter and radioautograph. The results of these determinations, Table 2 and Fig. 2, show that the cells of dolphin S-23 absorbed much more labelled protein than did those of S-25.

Samples of blood dried on filter paper strips were moistened by diffusion in four percent ammonium sulfate and then placed with their ends in the same solution (Fig. 3). When diffusion had started, equal sized drops of absorbed antiserum (one in eight dilution) were placed so that they could diffuse through the blood spots. After two hours the filter papers were allowed to dry at room temperature and the total radioactivity of the blood spots was determined by G-M counter. This experiment was repeated five times with replicated samples from each individual. The results are shown in Table 2. These agree very well with those obtained with intact cells in the first experiment.

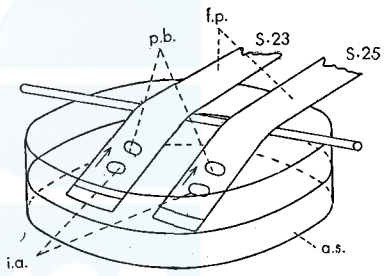

Fig. 3. Preparation of antigen-antibody reaction on filter paper, p.b.: spots of blood preserved by drying, i.a.: spots of iodinated antibody, f.p.: filter paper, a.s.: 4 percent ammonium sulfate.

CONCLUSION

These experiments show that it is highly probable that ^{131}I labelled antibodies can be used to distinguish individual antigenic differences in samples of dired blood. Such samples have great potential value in blood type studies on marine vertebrates where it may be difficult to preserve intact erythrocytes.

In order to expand these techniques for use in large scale investigations of fisheries problems, further research will be necessary on such matters as the extent of the variability introduced during the preparation of dried samples, as well as the general practicability of the method used.

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