was present. By the end of the 3 week postoperative period, normal healing was considered well underway (Figure 2), and there was, by then, no drainage from any of the suture sites, though the sutures were still freely movable.

One week before the scheduled release, the instrument package saddle was mounted onto the sutures to allow the animal time to adjust to it before adding the somewhat heavier (approximately 6 kg) instrument package itself. The animal occasionally rubbed the saddle against the side of the tank until the attachment was tightened to reduce free-play of the saddle as the animal swam. On the day before release, cracking of the polypropylene sutures was noticed, requiring their replacement with sutures of the same diameter composed of polyvinyl chloride coated stainless steel. These were found to be more pliable and stronger than the polypropylene.

At the last visual sighting of the animal on 7 April 1972, the instrument package was still securely attached despite the fact that, on several occasions, kelp had been seen trailing from it (J. S. Leatherwood, pers. comm.). At this time, we have no indication that this procedure has, in any way, compromised the ability of this animal to survive.

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MFR PAPER 1049

Some Hematologic Observations on the California Gray Whale

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ABSTRACT

Examination of the blood of the California gray whale, obtained shortly after its arrival at Sea World, San Diego revealed the following data: WBC-13.9 × 10^3/cubic mm; RBC-2.4 × 10^6/cubic mm; hemoglobin-10.0 g/100 ml; hematocrit-31 percent; MCV-128 μ^3; MCH-42.8 $\mu\mu$g; MCHC-32.4 percent. Hemoglobin electrophoresis showed a single hemoglobin band with a mobility similar to that of human hemoglobin F. The whale hemoglobin was 100 percent alkali resistant. No changes of this hemoglobin were seen on repeated analyses over the course of 12 months.

The capture of a young, female California gray whale, *Eschrichtius robustus*, in Scammon's Lagoon, and its maintenance in captivity at Sea World, San Diego for 12 months provided the opportunity for some hematologic studies which are to be reported here.

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ROUTINE BLOOD EXAMINATION

A heparinized blood sample obtained on 18 March 1971, one day after the arrival of the whale at Sea World, was brought to the Clinical Laboratories of University Hospital, University of California, San Diego. The blood was analyzed on a Coulter Counter,[1] Model "S", which allows the automatic simultaneous determination of cell counts, mean corpuscular volume (MCV), and hemoglobin content. The hematocrit, mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) are automatically computed from the three parameters measured (Pinkerton et al., 1970) The instrument is standardized twice daily and performs approximately 200 analyses per day for clinical purposes. The results were the following:

WBC-13.9 × 10^3/cubic mm
RBC-2.4 × 10^6/cubic mm
Hemoglobin-10.0 g/100 ml
HCT-31 percent
MCV-128 μ^3
MCH-42.8 $\mu\mu$g
MCHC-32.4 percent

A *blood smear* was prepared and stained by the automatic HEMA-TEK[2] technique, which employs a

[1]Coulter Electronics, Inc., Hialeah, Fla. References to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[2]Ames Company, Division of Miles Laboratories, Inc., Elkhart, Ind.

modified Wright-Giemsa stain, and examined by oil immersion microscopy.

The red cells were round, moderately anisocytotic ranging from 7.5-9.5 μ in diameter, and appeared well hemoglobinated with only occasional slight central pallor. An occasional red cell displayed polychromasia, and some rare Howell-Jolly bodies were seen. No nucleated red cells were encountered.

A white cell differential count was as follows:

Segmented neutrophils	63 percent
Band forms	19 percent
Metamyelocytes	< 1 percent
Monocytes	9 percent
Lymphocytes	8 percent

No eosinophils nor basophils were encountered. The lymphocytes were all of the large type. No small lymphocytes with the typically scant cytoplasm and dark staining nuclei were present. Twenty-one percent of the nuclei of the mature, segmented neutrophils had distinct "drumstick" appendages.

The thrombocytes appeared as round platelets, with diameters approximately one-third to one-half of those of the red cells. Their number, estimated from their frequency distribution on the smear in relation to the erythrocytes, was in the range of 300,000-350,000/cubic mm.

HEMOGLOBIN ELECTROPHORESIS

Hemoglobin electrophoresis was performed by the vertical acrylamide gel technique as described in detail elsewhere (Bierman and Zettner, 1967) (Nakamichi and Raymond, 1963). Briefly, a toluene hemolysate of the washed red cells is prepared and electrophoresed in Tris-buffer of pH 9.0 for 3½ hours at 120 ma. The acrylamide gel slabs are then stained with amido black and destained electrophoretically in 5 percent acetic acid.

The results are shown in Figure 1. The whale hemoglobin (Slots Nos. 1 and 7) migrated slightly slower than human hemoglobin A. The position of the band of the whale hemoglobin was indistinguishable from that where human hemoglobin F would be expected. No minor hemoglobin components equivalent to those found in human blood could be detected. The weakly stained band of much slower mobility, as shown in Figure 1, is a non-heme protein, as indicated by the failure of this protein band to react with benzidine when a freshly electrophoresed, unstained strip of the gel containing the whale sample was submersed in a benzidine and peroxide solution.

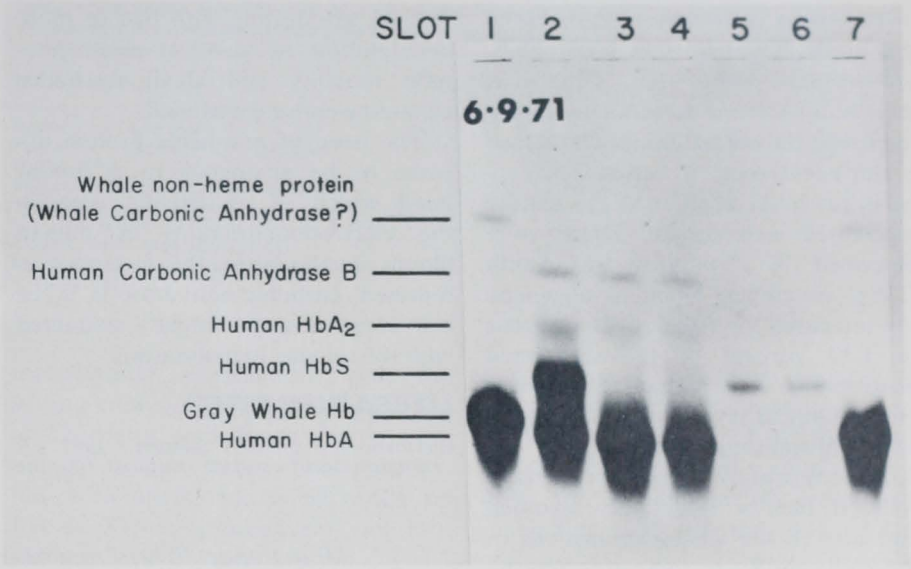

Figure 1.—Vertical acrylamide gel hemoglobin electrophoresis, Tris-buffer, pH 9.0. The gel contains the toluene hemolysates of the following: Slots No. 1-gray whale; No. 2-human with A-S trait; No. 3, 4-normal humans; No. 5,6-standards; No. 7-gray whale (same as slot No. 1). Original (sample application slots) at top. Cathode-top; anode-bottom.

The pattern of hemoglobin electrophoresis performed on blood samples obtained on 17 March and 27 April 1971, and 13 March 1972 was identical to that demonstrated here.

ALKALI DENATURATION

A quantitative alkali denaturation test performed on the toluene hemolysate by the method of Singer, Chernoff, and Singer (1951) revealed the whale's hemoglobin to be 100 percent alkali resistant. The alkali resistance of the hemoglobin was the same in all samples obtained over the course of 1 year, as listed above.

DISCUSSION

The values of the various red cell parameters, as reported here, are in fair agreement with those published by Lenfant (1969). Relative to most terrestrial mammals, the California gray whale appears to have lower red cell counts, hemoglobin concentrations, and hematocrits, although the MCV is considerably in excess of 100 μ^3. A proportional increase of the MCV of red cells with total body length of marine mammals of different species has been shown (Lenfant, 1969). Of interest is the finding of Lenfant (1969) of a high proportion of nucleated red cells in the gray whale. This is in distinct contrast to the complete absence of nucleated red cells in the blood samples examined here. It should be considered that the previous observations were apparently made on sick, wounded, dying, or dead animals; and that under these abnormal conditions, immature red cells may have been released into the circulation. The only indication of young red cells in our samples were the rare Howell-Jolly bodies and occasional polychromasia.

The white cells were remarkable in that no small lymphocytes, eosinophils, or basophils were seen. Otherwise, their numbers and percentages appear to be near the normal limits. Of interest is the occurrence of "drumstick" appendages in 21 percent of the mature segmented neutrophils. These were described by Davidson and Smith (1954) in human blood as a genetic sex indicator for females. They occur in 1-17 percent of the segmented neutrophils of all human females and are thought to represent the inactivated X-chromosome, analogous to the Barr body observed in most somatic cells. It can be reasonably assumed that also in the whale, drumsticks in the neutrophils are indicators for the female sex.

The uniform electrophoretic mobility of this gray whale's hemoglobin, characteristic of human hemoglobin F, is in accordance with the finding of others (Lenfant, 1969). Of further interest was the hemoglobin's resistance to alkali denaturation. However, no conclusions can be drawn from this coincidental sharing of two physical properties with human hemoglobin F as to functional or structural similarities between these two hemoglobins. The reasons for the alkali resistance of certain hemoglobin variants are poorly understood. In the human this is related not only to the presence of gamma chains in the hemoglobin molecule, but also to the structural relationships of the various chains to each other. For instance, Bart's hemoglobin, composed of four gamma chains, is only half as alkali resistant as hemoglobin F, which is a tetramer of two alpha and two gamma chains. The elucidation of the structure of the gray whale's hemoglobin depends on the full analysis of its amino acid sequence. Such an undertaking can also be expected to provide some evolutionary clues for the California gray whale.

From the evidence presented here, it appears that this species possesses only one type of structurally uniform hemoglobin, although the possibility that we are dealing with two or more hemoglobins of identical electrophoretic mobility and alkali resistance cannot be entirely excluded.

The band of non-heme protein appears to be analogous to a similar band which is consistently seen in the electrophoretograms of human bloods. In the latter, this is known to represent carbonic anhydrase B, a red cell constituent persistently extracted with the toluene hemolysates.

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MFR PAPER 1050

Some Coagulation Factors in Plasma from a California Gray Whale, *Eschrichtius robustus*

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ABSTRACT

A citrated plasma sample was assayed for some coagulation factors. The levels obtained were compared with those from some of the small toothed whales. Factor XII activity was very low in the gray whale sample, whereas toothed whales have none.

INTRODUCTION

Many people working with small odontocete whales in captivity have made the observation that whale blood has a prolonged clotting time. Since this observation was made two reports have described the lack of clotting Factor XII in blood in some of the smaller whales (Lewis, Bayer, and Szeto, 1969; Robinson, Kropatkin, and Aggeler, 1969). Another publication reports a prolonged clotting time of blood from other small whales; however, assays for Factor XII were not made (Ridgway, 1972). There were no reports of similar studies on blood from any baleen whale; hence this report on some studies on a plasma sample from a captive California gray whale, *Eschrichtius robustus*.