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STATUS OF SEROLOGICAL STUDIES ON MARINE MAMMALS IN NORWAY

by

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Introduction

Results of serological studies on seals and whales for identification of population units hav been discussed in previous reports (Nævdal 1966a, b, 1969a, b). Studies on whales, mainly the fin whale, were started in 1968 with analyses of blood proteins (hemoglobins and serum proteins). A rather limited material have since then been collected and analysed including specimens from fin whales, sperm whales, sei whales, minke whales and bottle-nose whales in 1971. Analyses of tissue extracts for identification of genetically controlled variations in enzymes have also been started. The present report gives preliminary results of blood protein analyses of whale species, and isoenzyme analyses of hooded seals and whales.

Material and methods

In Table 1 an account is given of collected and analysed whale blood samples.

Before 1970 all blood samples were collected on land stations after the whales were brought ashore. The samples collected in 1971 and the 19 specimens of fin whales from East-Greenland

in 1970 were collected on board the whaling boats immediately after the whales were shot.

The blood was centrifuged, sera pipetted off and cells and sera frozen separately. For analyses of isozymes of bottle-nose whales, a piece of muscle was cut out and frozen. The material was stored and shipped in frozen state until analysed at the Institute of Marine Research in Bergen.

Often part of the cells were ruptured before centrifugation, and consequently the sera contained considerable amounts of hemoglobins. This was especially evident for sera collected at land stations, and often had a bad effect on the results (see below).

The hemoglobins and serum proteins were analysed in combined starch and agar gel electrophoresis (Møller 1966), partly also in starch gel electrophoresis (Poulik 1957). Tissue extracts were prepared by grinding a piece of muscle with sand and water, and after centrifugation, the supernatant was subjected to electrophoresis. Extract of pancreas of hooded seals, collected for analyses of amylase (Nævdal 1969b), was prepared in the same way.

The gels were stained by general protein stains (Amidoblack 10B or Nigrosin) and with o-dianisidine for identification of haptoglobins. For identification of lactate dehydrogenase (LDH) and aspartate aminotransferase (AAT) in tissue extracts of bottle-nose whales and hooded seals, and in sera of fin whales, the gels were stained by histochemical staining procedures as described by Odense, Allan and Leung (1966).

Sera of fin whales have been sent to Universal Scientific Limited, London, for analyses by their Gradipore technique, but the results have not been received yet.

Results

Hemoglobins

Usually clear and well defined electrophoretic patterns were obtained by the hemoglobin analyses. However, individual variation which could be genetically controlled were not found. The hemoglobins of minke, fin and sei whales appeared to have identical electrophoretic mobility. The relative electrophoretic mobility of the hemoglobins of the species analysed is shown in Fig. 1. The only species which normally showed more than one hemoglobin pattern was the bottle-nose where one strong and one weaker component were invariably seen. Occasionally also a cathodic component occurred in fin whales, but this component probably represent denaturation products.

Serum proteins

The fin whale specimens from East-Greenland 1970 gave clear electrophoretic patterns of serum proteins. The individual patterns showed several individual variations, but the variations were not clear enough for a grouping of the specimens into well defined phenotypes. In Fig. 2 some of the observed patterns are outlined together with serum protein patterns of minke and bottle-nose whales.

The γ-globulins were seen at the cathodic side of the application point. The albumins with one prealbumin band showed the highest anodic mobility. Between the albumins and the application point six bands were located, Fig. 2. Additional bands occurred, but all very weak.

The three bands nearest to the albumins represent the free haptoglobins. In these bands clear intraspecific variations were seen. However, when hemoglobins are added to sera the haptoglobins bind hemoglobins and change their mobility. This is illustrated in pattern C and D in Fig. 2 where the same serum with and without hemoglobin is analysed. Only one of the

bands is present when sera contain hemoglobins. This band stains with o-dianisidine also when nearly invisible amounts of hemoglobins is present. Because only very few sera were completely free of hemoglobins, classification of the sera into well defined phenotypes on the basis of variations in the free hemoglobins was very difficult.

The sera of fin whale from other areas showed the same general patterns as the sample from East-Greenland, but due to a high degree of hemolysis, it was impossible to decide whether there could be qualitative variation of systematic value among these samples.

The serum samples of sei and sperm whales showed too much hemolysis to be used for description of patterns of serum proteins of these species.

Selected serum protein patterns of bottle-nose and minke whales are outlined in Fig. 2. In the bottle-nose two bands located behind the albumins showed intraspecific variation. One or both of these (tentatively called F and S) occurred in each individual, and in spite of limited material (21 specimens only) the distribution below indicate control by a two allele system.

	FF (F only)	FS (both)	SS (S only)
observed	2	12	7
expected Hardy-Weinberg distribution	3.0	9.9	8.1

No clear variation in the serum pattern of minke whales was seen, but only two specimens have been analysed and the results therefore are not conclusive.

Enzymes

Analyses have been started on lactate dehydrogenase (LDH) and aspartate aminotransferrase (AAT) and preliminary results of studies on pancreas amylase have been reported earlier (Nævdal 1969b).

The LDH patterns of fin whales and hooded seals were similar, Fig. 3, and they resemble the normal mammalian pattern with four or five different zones produced by combination of two different kinds of polypeptide chains (Manwell and Baker 1970, Numachi 1970). The pattern of the bottle-nose whale, however, differed quite markedly from the two other patterns, and in this species also individual variation was indicated, Fig. 3. The pattern to the right was seen in three out of 27 specimens analysed. Whether this pattern represent genetic variation cannot be told at present, but this can probably be verified when a larger material is analysed.

Only a few specimens of hooded seals and fin whales have yet been analysed for AAT, and they all gave only one zone of AAT intensity giving no indication of intraspecific variation.

Discussion

The hemoglobins of whales as well as of seals (Nævdal 1966b) have not shown genetically controlled variations at high frequencies, and they seem to be of little value as systematic tools on the subspecific level.

It is difficult to draw any general conclusion about the excistence of serum protein polymorphism in whales from the analysed samples. A great part of the material have been of poor quality when analysed and the results therefore are difficult to interpret. However, also in the fin whale sample which gave electrophoretograms of high quality, no well defined variations which could be used for identification of populations, were seen. Because of poor quality of the material, no conclusions about the sei and sperm whales can be drawn, and of minke whales only two specimens have been analysed. Only the bottle-nose whale have shown individual variation which seems to be genetically controlled, and although the two electrophoretic bands showing this variation, were rather poorly represented, identification of three phenotypes presented no real difficulty.

The material analysed is too limited to draw any conclusions on the occurrence of isoenzyme polymorphism in marine mammals. Individual variation in pancreas amylase have been observed (Nævdal 1969) but classification of the specimens on the basis of this variation was very difficult. The patterns of LDH of bottle-nose whales may represent genetic variation, but no proof can be given at present.

For analyses of serum proteins it is obviously important that the blood samples are collected soon after the animals are killed. If this precautions can be taken, it seems worth while to continue analysing blood samples, and especially it would be interesting to confirm the hypothesis of genetically controlled variations in the bottle-nose serum proteins. Also analyses of tissue isoenzymes will be continued. These characteristics seems to withstand storing much better than do serum proteins, and therefore material are much easier obtained.

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Table I. Blood samples of whale species analysed for intraspecific variation in blood proteins.

Area	Year	Minke	Sei	Fin	Sperm	Bottlenose
Canada (east coast)	1968	—	—	7	—	—
Iceland	1968	—	3	23	28	—
Canada (east coast)	1969	—	—	12	—	—
Norway (Tromsø)	1969	—	—	2	2	—
Norway (Tromsø)	1970	—	—	4	—	—
East - Greenland	1970	—	—	19	—	—
Canada (Labrador)	1971	—	—	—	—	21
Norway (Vestfjorden)	1971	2		—		—

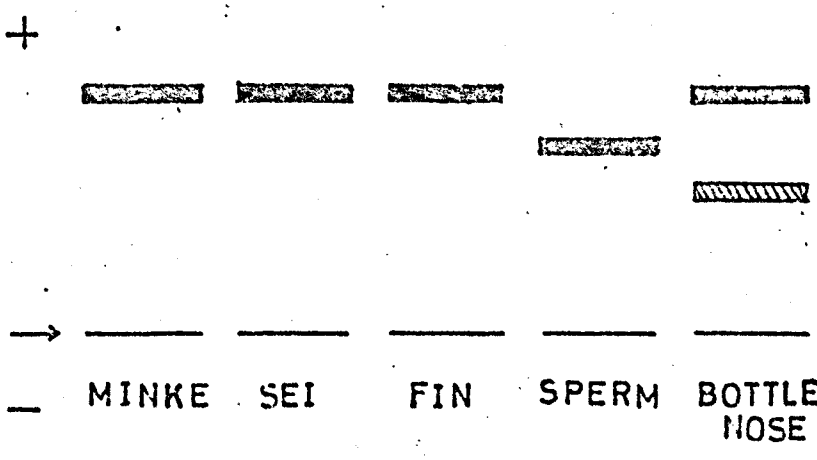

Fig. 1. Hemoglobin pattern in four whale species by combined starch and agar gel electrophoresis. Legend in this and the following figures: Filled in bars: Strong bands. Hatched bars: Moderately stron bands. Single lines: Faint bands. Arrow indicates the point of application.

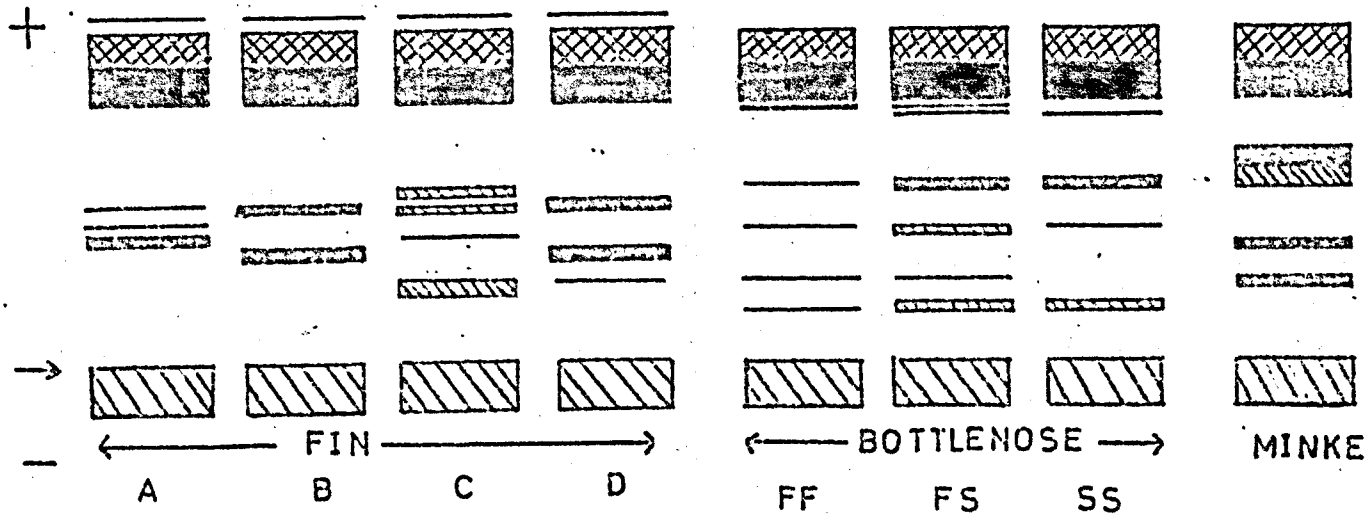

Fig. 2. Serum protein patterns in three whale species. Legend: Fig. 1. For further explanation, see text.

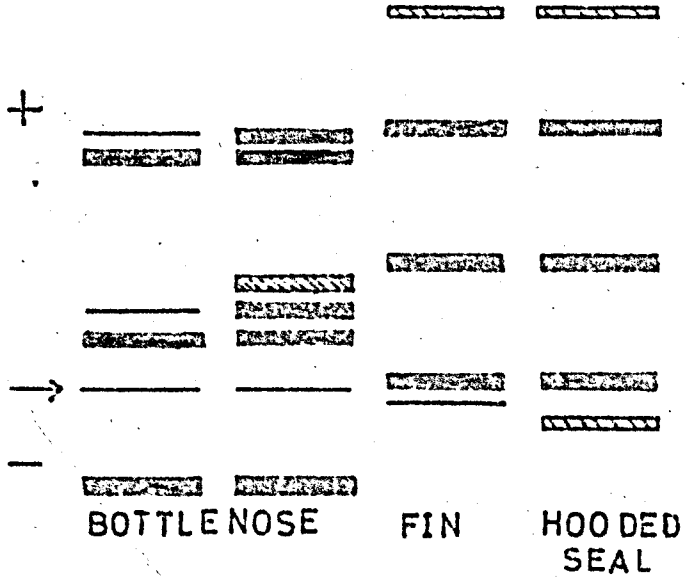

Fig. 3. Patterns of lactate dehydrogenase (LDH) in three species of marine mammals by combined starch and agar gel electrophoresis. Legend: Fig. 1.