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A PRELIMINARY REPORT ON ELECTROPHORETIC STUDIES ON HERRING SERUM PROTEINS

by

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Abstract

Sera from more than 1 000 specimens of herring from the Norwegian Coast and the North Sea have been analysed in combined starch-/agar-gel electrophoresis at pH 9.0.

Great variations in the serum protein patterns were observed. Especially the transferrin variations (identified by Fe^{59}-autoradiography) were conspicuous. Two transferrin components, either occurring alone or together, were found.

Also two main albumin components were observed, and they too occurred alone or together. However, the determination of the albumin types were often somewhat unreliable.

The variations both in the transferrins and the albumins might be explained by a hypothesis of genetic control involving two allelomorphic genes.

Variations were also observed in other serum proteins. These proteins, however, occurred only at low concentration in the sera and therefore appeared on the electrophoretograms as bands which were too weak or too diffuse for proper classification.

Considerable variations were found in frequencies of the transferrin components in samples from different areas. The variations in samples seemed less evident for the albumin components. The geographical variations will be more completely dealt with in a later report.

Introduction

Electrophoretic investigations on blood proteins have been carried out on several species of fish in order to reveal characteristics to be used in taxonomic studies on the sub-specific level (Sick 1961, Sinderman and Honey

1963, Møller 1965, Møller and Nævdal 1966), and especially proved successful for cod haemoglobin types (Sick 1965 a, b, Frydenberg et al. 1965). At the Institute of Marine Research haemoglobins and serum proteins from herring have been subjected to electrophoresis. Evidently ontogenetic variations were present in the herring haemoglobins. However, ontogenetic variations have been described in details by Wilkins and Iles (1966), who found that the variations were assosiated with lenth and sexual development. Consequently it seems doubtful to use haemoglobin types in taxonomic investigations of this species.

Consequently the serological work has here been concentrated upon electrophoresis of serum proteins, and especially have the serum transferrins been paid attention to. This report deals mainly with description of the serum protein types and with attempt to reveal the genetic basis of the variations. The geographical variation is not dealt with in detail here, but will be covered by a later report.

Material and methods

The blood was taken by cardiac puncture within few minutes after the fish was killed, then transferred into 2 ml glass-tubes and allowed to clot. The tubes were packed on ice in thermobottles, and as soon as possible sent to the laboratory. After centrifugation, the sera were pipetted off and analysed fresh or after storage for some days or weeks in a deep freezer. No differences were found between fresh sera and sera which had been stored frozen for a few weeks, but the fresh sera usually gave results of higher technical quality. Storage of sera for periods of three months or more seemed to damage the proteins to some degree and made the results unreliable.

Combined starch-/agar-gel electrophoresis at pH 9.0 (Sick 1965 a, Møller 1965) on microscopic slides was applied. The proteins were stained in Amidoblack 10 B or Nigrosine. Autoradiography according to Giblett, Hickman and Smithies (1959), modified for this type of electrophoresis by Møller (1965), was carried out for identification of serum transferrins. Staining with o-dianisidine (peroxidase activity) was applied to detect haptoglobin/haemoglobin complexes.

Localities of sampling, numbers in samples, dates of sampling, and fishing gears follow from Table I.

Results

Description of the serum protein electrophoretograms.

By combined starch-/agar-gel electrophorsis at pH 9.0 all the serum proteins moved towards the anode. Electrophoretograms obtained by ana-

lysis of sera containing some haemolysate are outlined in Fig. I. By the present method, the three strong haemoglobin fractions described by Wilkins and Iles (1966), using agar-gel electrophoresis at pH 7.2 were found on the anodic side of the point of application, and their somewhat weaker fraction 4 on the cathodic side of this point. The haemoglobin patterns are generalized in Fig. I, but the three strong fractions were never found in the same specimen.

The herring serum transferrins, identified by autoradiography, had an anodic mobility greater than the haemoglobins, but less than any of the other serum proteins.

Two transferrin bands were commonly found. They were called Tf A and Tf B, the first having the greater anodic mobility. Each specimen might posess one or both of the transferrins, and accordingly three transferrin types (called Tf AA, Tf AB, and Tf BB) were revealed. Tf AA and Tf BB contained only Tf A or Tf B respectively and Tf AB contained both the transferrins.

As indicated in Fig. I, specimens which contained only one strong transferrin band, often showed a faint band at the position of the lacking transferrin band. Such specimens were classified as if the weak band was absent (Tf AA or Tf BB phenotypes). Since the faint bands might vary in strength, it was sometimes difficult to distinguish these specimens from those belonging to the phenotype Tf AB. Usually this problems were solved by repeated analysises.

In som specimens indications of other transferrin components were found. One band was seen just behind Tf A in a few specimens. However, this band was difficult to distinguish from the Tf A-band. Its frequencies therefore were unreliable and consequently of little value in population studies. A fourth band, called Tf A', probably also represented a rare transferrin component (Fig. I e). The two components were not tested autoradiographically since the sera in which they occurred were not available when the tracing experiments were made. The latter components were classified together with Tf A.

A broad and diffuse band of somewhat greater mobility than Tf A was sometimes seen (Fig. I d). Its position, and especially its strength, might differ somewhat, and as it always was located near Tf A, it made the determination of the transferrin types difficult. This band probably represented the "mature female proteins" (de Ligny, personal communication), as it was only found in females of maturity stages V and VI. However, although the band was associated with the maturing process, it was only present in part of the females of these maturity stages.

Between the transferrins and the albumins several bands were seen on the electrophoretograms. Although great individual variations were observed, these bands were too weak or too diffuse to form the basis of classifying the individuals into well defined groups. Some of the patterns are outlined

in Fig. I. Since one of the mentioned bands was stained with o-dianisidine, it probably represent haemoglobin/haptoglobin complexes.

Two main albumin components could be distinguished, and one or both of these were present in each specimen (Fig. I). The faster moving component (anodic mobility) was called Alb F, and the slower moving, Alb S. The three resulting phenotypes were called Alb FF, Alb FS, and Alb SS according to which of the albumin components they posessed. However, the differences between the albumin types were often not significant, especially between the types Alb FS and Alb SS. Due to these difficulties the albumin type determinations were not quite reliable.

Outside the albumins one or two faint prealbumin bands were observed.

Geographic variation

Table 1 gives the observed frequencies of Tf A in the analysed samples. It follows from the table that the highest frequencies of Tf A was found in the samples of young herring from inshore waters of Western Norway. Samples no 1 and 3 showed approximately the same frequencies, but sample no 2 deviated somewhat. It should be noted that the distribution of phenotypes in this sample was not in accordance with the expected Hardy-Weinberg distribution (see below), which might indicate that the sample contained individuals from two populations in which Tf A occurred at different frequencies.

In the samples of adult Norwegian spring spawners the frequencies were somewhat lower than in the 0-group of herring from the coast of Western Norway, and no significant differences were observed between the samples from the southern (off Møre) and northern (off Lofoten) spawning grounds.

In the two samples of autumn spawners from the North Sea, the frequencies of Tf A were significantly lower than for the spring spawners, but the two samples did not agree very well. It is suggested that these two samples consisted of Bank herring and Kattegat autumn spawners in unknown proportions. Vertebral numbers of sample 7 indicated, however, a higher proportion of Kattegat autumn spawners, which may explain the observed differences of Tf A.

The frequency of the spawning herring from inshore water of Western Norway (sample no 6) deviated from these obtained for the spawning herring further north on the coast. It should be noted that these two populations also deviated in age composition, scale pattern, and growth rate (Haraldsvik 1966).

However, the geographic variations of the frequencies in Tf-components will be dealt with when further material have been collected from the herring populations along the Norwegian Coast and the North Sea.

Only small variations were found in the frequencies of the albumin components

Where albumin types could be determined with a reasonable degree of reliability, Alb A was observed at a frequency of about 0.40 in the samples.

Discussion

Two allelomorphic genes (called $\underline{Tf}^A$ and $\underline{Tf}^B$), each controlling one transferrin component, would explain the individual variation in the herring serum transferrins. The resulting genotypes are designed $\underline{Tf}^A/\underline{Tf}^A$, $\underline{Tf}^A/\underline{Tf}^B$, and $\underline{Tf}^B/\underline{Tf}^B$. This corresponds to the transferrin variations in cod (Møller 1963) and in some other gadoid fishes (Møller and Nævdal 1966). Assuming the above gene combinations, the gene frequencies are calculated from the observed distributions of the transferrin types in each sample. Observed distributions of phenotypes are compared with the expected Hardy-Weinberg distributions of genotypes. In most cases there are good agreement between observed and expected distributions, and the deviations are not significant (except in sample no 2). Consequently, genetical control by two allelomorphic genes may well be accepted to explain the herring transferrin variations.

The rare occurring transferrin components are believed to be controlled by other alleles belonging to the same system. However, too few cases with these components are observed to test the material with statistical methods.

The presence of weak components in addition to the stronger transferrin components do not necessarily cause any difficulties in explaining the transferrin variations in herring. Simultanous variations of a major and a minor component has been reported for the serum transferrins in mouse (Schreffler 1960), and a similar mode of inheritance may also be the case in herring. However, the minor components of the mouse transferrins were invaryably present, whereas in herring they might vary in strength and even be lacking. This indicates a more complicated genetic control by modifying genes, or dependence upon noninherited factors.

The albumin variations might be genetically controlled in a similar way; two alleles (called $\underline{Alb}^F$ and $\underline{Alb}^S$), each controlling one main albumin component. The observed distribution of phenotypes and the expected Hardy-Weinberg distribution of supposed genotypes (called $\underline{Alb}^F/\underline{Alb}^F$, $\underline{Alb}^F/\underline{Alb}^S$, and $\underline{Alb}^S/\underline{Alb}^S$) are in fairly good agreement. The hypothesis may therefore be correct, which eventually will be confirmed by analysis of additional material. The albumin variation found in herring and its supposed genetical control are similar to the albumin variations in man (Knedel 1958, Efremow and Brænd 1964), chicken (McIndoe 1962), and horse (Brænd 1964).

At present no explanation of the other serum protein variations can be given; the protein bands in all cases being too weak for proper classification of the individuals.

The results of these preliminary investigations indicate that serum transferrin polymorphism may be useful in herring sub-species taxonomy. The variations among populations seems to be significant, but further observations are needed

before the given results can be verified. Although only small differences were observed among the samples of different populations, increased numbers of observations may give albumin variations which can be used in population studies.

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Table I. Herring samples analysed for transferrin types and observed frequencies of Tf A.

Sample no.	Locality of sampling	Fishing gear	Date	Number	Frequency of Tf A	
1.	Håvik i Fusa, Western Norway (inshore waters)	purse seine	5.10.1965	78	0.88	0-group
2.	Fensfjorden " " " "	" "	15.10.1965	98	0.70	"
3.	" " " " "	" "	17.10.1965	90	0.87	"
4.	Buagrunnen, Off Møre, North-Western Norway	" "	10.-11. 2.1966	337	0.77	Norwegian spring spawners
5.	Røstbanken, Lofoten, Northern Norway	" "	10. 3.1966	109	0.78	"
6.	Fanafjorden, Western Norway (inshore waters)	net	14. 4.1966	113	0.60	Spring spawners
7.	Egersundbanken, North Sea	purse seine	24.-25. 5.1966	152	0.27	Autumn spawners
8.	" " "	" "	27. 6.1966	78	0.46	"

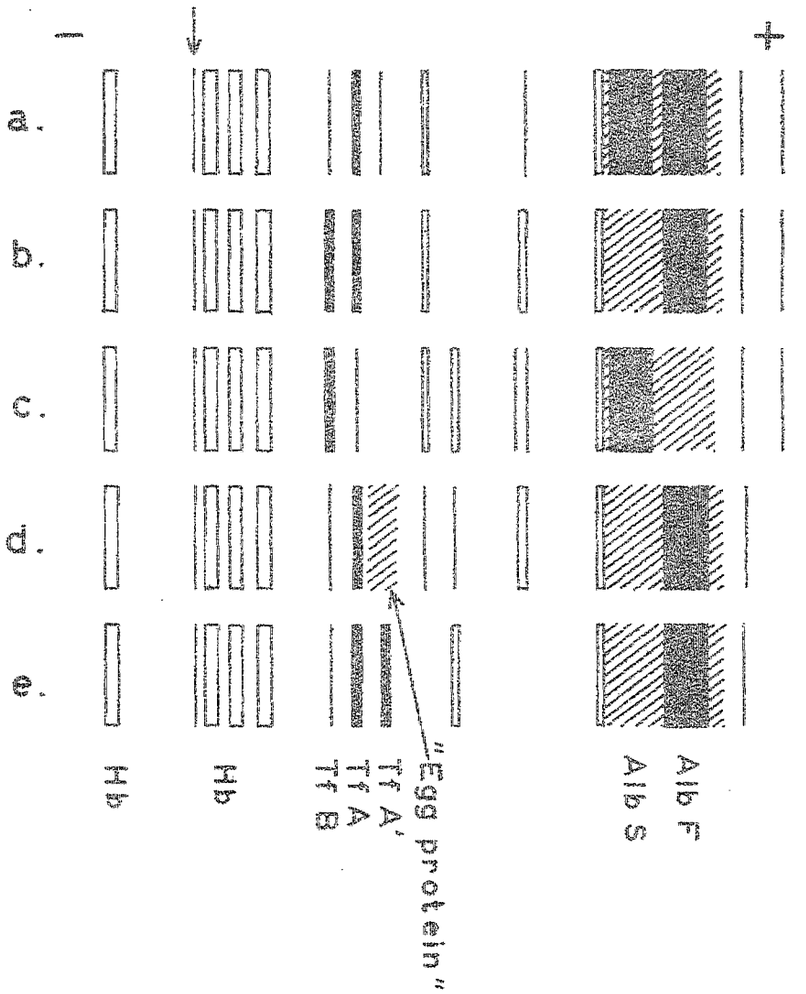

Fig. 1. Outline of herring serum protein electrophoretograms obtained by combined starch-/agar-gel electrophoresis at pH 9.0 for 90 minutes. The position of the various haemoglobin components are indicated.

Legend: Filled in bars: Strong bands. Open bars:Moderately strong bands. Single lines: Weak bands, and Cross hatches: Diffuse stained areas. Arrow indicates the point of application.