

STUDIES ON BLOOD PROTEINS IN HERRING

By

GUNNAR NÆVDAL

Institute of Marine Research, Bergen

INTRODUCTION

An investigation on blood proteins of herring, *Clupea harengus* L., was started in 1965 in order to find characteristics to be applied on the problem of identification of stock units. As part of this programme a report on serum esterase polymorphism has been published (NÆVDAL 1969). The present report deals with the electrophoretic analyses of hemoglobins and serum proteins. A complete description of ontogenetic variations in herring hemoglobin has been published (WILKINS and ILES 1966), and in the present report most attention has therefore been paid to the serum proteins.

MATERIAL AND METHODS

Blood sampling, treatment of blood and sera, analyses by electrophoresis, staining of proteins, autoradiography etc. were carried out in the same way as described for investigation on sprat (NÆVDAL 1968). PARKER and BEARN (1963) and CANN (1966) found multiple electrophoretic zones rising from protein-buffer interaction in the presence of boric ions. The buffer used in the present routine analyses contained boric acid, and to find out whether boric ions had any influence upon the protein zones, part of the material was analyzed by a buffer without boric acid but otherwise under identical conditions.

The type of herring investigated, date and locality of sampling and numbers in the samples are listed in Table 1. Age, sex, vertebrae numbers and otolith and scale type have been determined for part of the material. The specimens of samples 9 and 10 were caught as 0-group in August 1965 and separated into two batches which were kept isolated in tanks under simulated North Sea and Barents Sea temperature conditions (HARALDSVIK, personal communication).

Contribution given in honour of Gunnar Rollefson at his 70th birthday.

RESULTS AND DISCUSSION

BIOLOGICAL VARIATION

Hemoglobin

The results of the hemoglobin analyses were, except for insignificant differences, in accordance with the ontogenetic variations described by WILKINS and ILES (1966). The relationship between hemoglobin types and growth may be different in the various populations, but as long as this is not completely understood, hemoglobin types cannot be used for identification of stock units. Therefore a further discussion of the hemoglobin types have been omitted.

Serum proteins

All serum proteins moved towards the anode at pH 9.0. Considerable variations were noted in several groups of proteins. A representative selection of protein patterns (electrophoretograms) are shown in Fig. 1.

The serum transferrins, identified by autoradiography, had less anodic mobility than any other serum proteins. Two transferrin bands were common. They were named Tf A and Tf B, the first had the greatest anodic mobility. Each specimen might possess one or two of the transferrin bands, and the phenotypes were named Tf AA, Tf AB and Tf BB according to the components they possessed.

Specimens which contained a single strong transferrin band only, often showed a faint band at the position of the lacking transferrin band

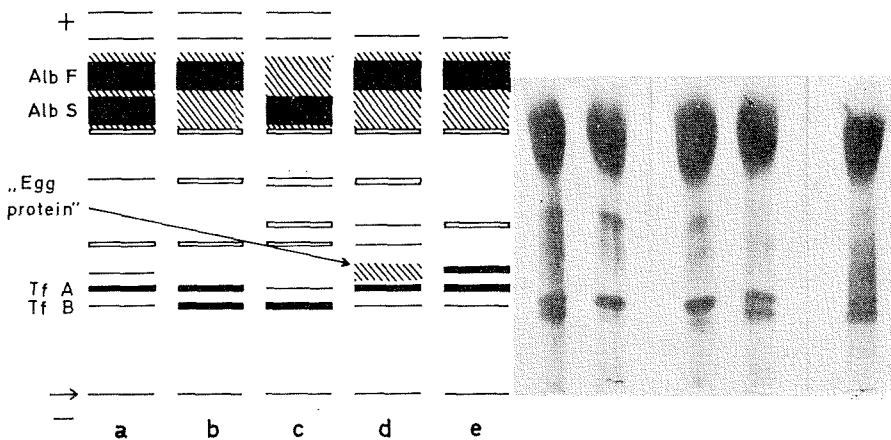


Fig. 1. Outline of serum protein patterns in herring obtained by combined starch and agar gel electrophoresis at pH 9.0, and photograph of electrophoretograms obtained by routine analyses. Filled in bars: Strong bands. Hatched bars: Moderately strong bands. Single lines: Faint bands. The point of application is indicated by the smaller arrow. From left to right the photographed types are: Tf AB, Tf AA, Tf AA, Tf AB, and Tf BB.

(Fig. 1). These specimens were classified as if the weak band was absent (Tf AA or Tf BB phenotype). The faint bands varied in strength among specimens and sometimes even between repeated analyses of the same specimen. The determination of the transferrin phenotypes were consequently to some degree unreliable.

A few specimens had one band close to Tf A at the cathodic side. A fourth band, seen on the anodic side of Tf A in a few specimens, probably represented a second rare transferrin component (Fig. 1e). The two rare bands were not tested autoradiographically since sera were not available when the tracing experiments were made, and they were both classified together with Tf A when calculating frequency distributions.

Presence of great amounts of hemoglobins in the serum slowed down the mobility of the transferrin molecules, especially Tf A, and consequently several specimens might be incorrectly determined as Tf BB. The relation between hemoglobins and transferrins was not clear, but the transferrins did not stain with *o*-dianisidine and consequently had no hemoglobin-binding capacity. Evidently sera containing considerable amounts of hemoglobins could not be used for determination of transferrin types.

Freezing and thawing of sera had no influence on the mobility of the transferrins or other serum proteins. However, a prolonged storage of sera in deep freezer had a similar effect on the transferrins as the presence of hemoglobins, namely reduced anodic mobility, making the type determination unreliable.

A broad and diffuse band was found on the anodic side of Tf A in females near spawning. Position and strength differed, but it was always located near Tf A, and therefore the classification of transferrin types in these cases was difficult. This band probably represented the "ripe female protein" observed in species of the genus *Oncorhynchus* (TSUYUKI and ROBERTS 1966) and the rainbow trout, *Salmo gairdneri*, (THURSTONE 1967).

Several protein bands were seen between the transferrins and the albumins. Although great variations were observed, these bands were too weak or too diffuse to form the basis of classifying the specimens into well-defined groups. Some of these patterns are outlined in Fig. 1. Since one of the mentioned bands stained with *o*-dianisidine, it probably represented haptoglobin/hemoglobin complexes.

Two main albumin components could be distinguished. One or both were present in each specimen (Fig. 1). The faster moving component was named Alb F and the slower Alb S. The three phenotypes were named Alb FF, Alb FS and Alb SS. The differences between the albumin types were often insignificant, and accordingly the albumin type determinations could only be accomplished in a few samples. In front of the albumins one or two faint pre-albumin bands were observed.

Table 1. Observed distributions (obs) of transferrin types in herring compared to expected Hardy-Weinberg distributions (exp).

Sample no.	Locality and date of sampling		Indications of sample		Transferrin groups			No.	q_A
					Tf AA	Tf AB	Tf BB		
1	Håvik i Fusa, Hordaland	5 Oct. 65	O-group	obs	60	17	1	78	0.88
				exp	60.4	16.5	1.1		
2	Fensfjorden, Hordaland	15 Oct. 65	O-group	obs	57	24	17	98	0.70
				exp	48	41.2	8.8		
3	Fensfjorden, Hordaland	23 Oct. 65	O-group	obs	86	20	4	110	0.87
				exp	83.3	24.9	1.9		
4	Buagrunden, off Møre	10-11 Febr. 66	Adult Norwegian spring spawners	obs	196	124	17	337	0.77
				exp	199.8	119.4	17.8		
5	Røstbanken, Lofoten	10 March 66	Norwegian spring spawners	obs	68	33	8	109	0.78
				exp	66.3	37.4	5.3		
6	Fana fjorden, Hordaland	14 April 68	Adult spring spawners	obs	43	50	20	113	0.60
				exp	40.7	54.2	18.1		
7	57° 40' N, 04° 30' E North Sea	24-25 May 66	Autumn spawners	obs	14	55	83	152	0.27
				exp	11.1	59.9	81.0		
8	58° 00' N, 05° 00' E North Sea	27 June 66	Autumn spawners	obs	17	38	23	78	0.46
				exp	16.5	38.8	22.7		
9	Lysefjorden, Hordaland	18 Aug. 66	See text	obs	12	51	35	98	0.38
				exp	14.2	46.2	37.7		
10	Lysefjorden Hordaland	23 Aug. 66	See text	obs	12	46	38	96	0.36
				exp	12.4	44.2	39.3		
11	Myking, Hordaland	April 67	Adult spring spawners	obs	37	33	13	83	0.64
				exp	34	38.3	10.8		
12	Duesund, Mastj. Hordaland	12 June 67	1-group	obs	69	25	2	96	0.85
				exp	69.4	24.5	2.2		
13	Halifax, N.S. Canada	June 68	Adult	obs	74	20	4	98	0.86
				exp	72.5	23.6	1.9		

None of the observed variations could be correlated with age or sex, except for the "ripe female protein".

The variations also occurred when boric acid was omitted in the buffer. Therefore the observed variations cannot be due to a protein-buffer interaction caused by boric ions. However, it is possible that other substances in the buffer or medium may interact with the proteins and thus cause artificial multiple zones.

Two allelomorphic genes (named Tf^A and Tf^B), each controlling one transferrin component, would explain the intraspecific variation in the herring serum transferrins. This corresponds to the transferrin variation in cod (MØLLER 1966) and in some other gadoid fishes (MØLLER and NÆVDAL 1966). The observed distributions of transferrin types in herring are shown in Table 1, and assuming the above gene combinations, gene frequencies were calculated from the observed distribution in each sample. Good agreements between observed and expected distributions were found in most cases. The deviations were not statistically significant, except for sample 2. This sample, which showed an excess of hypothetical homozygotes compared to expected numbers, might represent a mixture of two or more populations. Consequently, control by two allelomorphic genes may be accepted as an explanation of the herring transferrin variations.

The rare transferrin components are believed to be controlled by other alleles belonging to the same genetic system. However, because these components occurred only in a few specimens, this hypothesis could not be tested statistically. 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. Simultaneous variation of a major and a minor component has been reported for the serum transferrins in mouse (SCHREFFLER 1960), and the herring transferrins may be controlled in a similar way. However, the minor components of the mouse transferrins were invariably present, whereas in herring they might differ in strength and even be lacking. This indicates a more complicated genetic control by modifying genes or dependence upon non-inherited factors.

The albumin variations may also be controlled by two alleles, each controlling one main albumin component. In the few samples where the albumin types could be determined with a reasonable degree of reliability, fairly good agreement was found between observed and expected distributions. Consequently, the albumin variation in herring and its control may be similar to the albumin variation in man (KNEDEL 1958, EFREMOW and BRÆND 1964), chicken (McINDOE 1962), and horse (BRÆND 1964).

No hypothesis of genetic control of the other serum protein variations can be put forth due to the protein bands in all cases being too weak for proper classification.

GEOGRAPHICAL VARIATION

Assuming that the hypothesis of genetic control of the transferrin types in herring is correct, their frequency distributions may be used for identification of population units. The variations in the other blood proteins are at present not revealed clearly enough to be of any value for this purpose. Table 1 gives observed distributions of transferrin types and the frequencies (q_A) of the hypothetical gene Tf^A , taken as a characteristic sample parameter. All these samples have been analyzed fresh or fresh frozen, and sera with considerable amounts of hemoglobins have been omitted. Due to difficulties caused by the weak transferrin zones, statistical methods for comparing samples have not been applied. However, the considerable variations of q_A among samples may allow some tentative conclusions.

The highest value of q_A was found in some samples of young herring from inshore waters of western Norway (samples 1, 3 and 12). Sample 2 collected in the same area and approximately at the same time as sample 3, showed somewhat lower q_A -value, and the distribution of phenotypes in this sample was not in accordance with expected Hardy-Weinberg distribution, indicating a mixture of populations differing in q_A . The q_A -values of the samples 9 and 10 also indicate that herring of different origin is populating the inshore waters of western Norway. The herring in these samples had been caught in inshore waters south of Bergen and kept for one year at the Institute of Marine Research. These two samples of herring, exposed to different temperature conditions, showed nearly the same q_A -values. The values were much below the values of samples 1, 3 and 12, but similar to those observed in samples of North Sea autumn spawners (samples 7 and 8). The mean vertebrae number and otolith characters indicated that these young herring originated from North Sea autumn spawners (HARALDSVIK, personal communication).

No significant differences were observed between the samples from the southern (off Møre) and northern (off Lofoten) spawning grounds of the Norwegian spring spawning herring, but differences should not be expected since the northern group was recruited from the southern area in 1959-61 (DEVOLD 1968). The q_A -values of these samples were higher than for samples of mature spring spawning herring from inshore waters of western Norway south of Møre (samples 6 and 11). Differences have been found in age composition, scale pattern and growth rate between Norwegian spring spawning herring and spring spawners from inshore

waters of western Norway (HARALDSVIK 1968). Samples 6 and 11 also differed from samples of young herring in the same fjords indicating that the adult and young herring in the same area may originate from different populations (local populations, spawning migrations of other populations and eggs and larvae from offshore waters).

In the two samples (7 and 8) of autumn spawners from the North Sea the q_A -values were significantly lower than for the other samples, but the q_A -values in these two samples did not agree. It is suggested that the samples consisted of a mixture of different groups of autumn spawners and this may explain the observed differences.

The sample from Canadian waters showed that variations in serum transferrins are present also in herring in the west Atlantic. The same phenotypes occurred as far as could be stated by the present electrophoretic methods, indicating control by homologous genes. The sample showed a gene frequency near the highest observed in samples from Norwegian waters.

SUMMARY

1. Hemoglobins and serum proteins of herring have been analyzed by agar gel electrophoresis (hemoglobins) and combined starch and agar gel electrophoresis (serum proteins). The material comprises twelve samples from the Norwegian coast and the North Sea and one sample from the east coast of Canada, a total of 1,546 specimens.
2. The results of the hemoglobin analyses were in general agreement with the ontogenetic variation described by WILKINS and ILES (1966).
3. Intraspecific variation was noted in the transferrin components. Three common transferrin types were found and assumed to be controlled by two allelomorphic genes. No relation to age or length was found. Presence of additional weak components complicated the type determination. Hemoglobins in the sera as well as prolonged storage made the type determination unreliable.
4. Observed albumin variations could be explained assuming control by two alleles, while observed variations in other serum protein groups were too weak or too diffuse for classification. A broad and diffuse band was found on the electrophoretograms from ripe females.
5. Considerable variations in the frequencies of the genes supposed to control the transferrin types were observed among samples. The type determinations were in some specimens complicated, but the observed variation were greater than what could be explained by incorrect type determination or sampling error. The variation therefore probably represent real differences among population units.

ACKNOWLEDGEMENT

My thanks are due to Dr. D. Møller, Bergen, and cand. real. S. Haraldsvik, Bergen, for valuable discussion and for sampling of part of the material. Thanks are also due to cand. real. P. Øynes, Bergen, to several fishermen and to staff of Noregs Sildesalslag for assistance in obtaining samples, and to Dr. P. H. Odense, Halifax, who kindly sent me the sera from Canadian waters.

REFERENCES

- BRÆND, M. 1964. Serum types of Norwegian horses. *Nord. VetMed.*, 16: 363-373.
- CANN, J. R. 1966. Multiple electrophoretic zones arising from protein-buffer interaction. *Biochemistry*, 5: 1108-1112.
- DEVOLD, F. 1968. The formation and disappearance of a stock unit of Norwegian herring. *FiskDir. Skr. Ser. HavUnders.*, 15: 1-22.
- EFREMOW, G. and BRÆND, M. 1964. Serum albumin: Polymorphism in Man. *Science, N.Y.*, 146: 1679-1680.
- HARALDSVIK, S. 1968. Relation between the Norwegian spring-spawning stock and the spring-spawning group of herring in the north-eastern North Sea. *FiskDir. Skr. Ser. HavUnders.*, 14: 183-198.
- KNEDEL, M. 1958. Über eine neue vererbte Protein-anomalie. *Clinica chim. Acta*, 3: 72-75.
- MCINDOE, W. 1962. Occurrence of two plasma albumins in the domestic fowl. *Nature, Lond.*, 195: 353-354.
- MØLLER, D. 1966. Serum transferrin in cod. *FiskDir. Skr. Ser. HavUnders.*, 14: 51-60.
- MØLLER, D. and NÆVDAL, G. 1966. Serum transferrin in some gadoid fishes. *Nature, Lond.*, 210: 317-318.
- NÆVDAL, G. 1968. Studies on hemoglobins and serum proteins in sprat from Norwegian waters. *FiskDir. Skr. Ser. HavUnders.*, 14: 160-182.
- 1968. Studies on serum esterase in herring and sprat. *FiskDir. Skr. Ser. HavUnders.*, 15: 83-90.
- PARKER, W. C. and BEARN, A. G. 1963. Boric acid-induced heterogeneity of conalbumin by starch-gel electrophoresis. *Nature, Lond.*, 199: 1184-1186.
- SCHREFFLER, D. C. 1960. Genetic control of serum transferrin type in mice. *Proc. Nat. Acad. Sci. U.S.*, 46: 1378-1384.
- THURSTONE, R. V. 1967. Electrophoretic patterns of blood serum proteins from rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.*, 24: 2169-2188.
- TSUYUKI, H. and ROBERTS, E. 1966. Inter-species relationships within the genus *Oncorhynchus* based on biochemical systematics. *J. Fish. Res. Bd. Can.*, 23: 101-107.
- WILKINS, N. P. and ILES, T. D. 1966. Haemoglobin polymorphism and its ontogeny in herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). *Comp. Biochem. Physiol.*, 17: 1141-1158.

Received 30 November 1968

Printed 10 November 1969