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VARIABILITY OF THE ELECTROPHORETIC
PATTERNS OF BLOOD SERUM PROTEINS
IN A WHITEFISH, COREGONUS
OLUPEAFORMIS, POPULATION

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ABSTRACT

VARIABILITY OF THE ELECTROPHORETIC PATTERNS OF BLOOD SERUM PROTEINS IN A WHITEFISH, COREGONUS CLUPEAFORMIS, POPULATION

By Henry E. Boone

If workers are to accept species-specificity of electrophoretic serum protein patterns for use in understanding phylogenetic relationships, a knowledge of the manner in which these proteins may vary should be realized. A review of the conditions that cause serum proteins to vary in fish and the results of an electrophoretic study of the serum proteins in a whitefish, Coregonus clupeaformis, population was done.

A Spinco-Beckman Model-R paper electrophoresis system was used to separate the whitefish serum into its various protein components. The whitefish blood was obtained by cardiac puncture, allowed to clot, centrifuged, and the serum was separated and frozen so as to be analyzed at a later date. An analysis of variance was done to determine if there were any differences amongst age-groups III, IV, V, and IX for percentage composition of similar serum protein components.

The serum protein electrophoretic patterns revealed significant increases by age for two serum globulin components.

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INTRODUCTION

A. Purpose of Study

It has been known for more than 60 years that certain proteins characterize every species of plant and animal and that phylogenetic relationships are reflected in protein structure. The first application of this fact to taxonomic studies was by Nuttall (1901; 1904) who used the precipitin reaction of immune sera to test degrees of relationship in over 500 species of animals.

The protein components of animal blood serum are responsible for transport of the red cells, supply of all the materials for the body's metabolic requirements, defense against invasion and injury, maintenance of body pH and osmotic pressure and regulation of cellular activity and function. These functions of the blood serum are common to all animals and thus certain physiological features of the various blood serum protein molecules are retained in each species even when molecular changes may occur from species to species.

A conservative taxonomic character is one which preserves evidence of evolutionary relationships. Even though serum proteins are species-specific and of a conservative taxonomic nature to allow for interpretation of phylogenetic relationships, there is a large amount of evidence in the literature concerning variability in these proteins in members of populations of fish and other animals. Before

the serum proteins in fish can be used for phylogenetic studies, a knowledge of the manner in which these proteins may vary should be realized. The purpose of this study is to assemble some of the evidence found in the literature of the conditions that cause the serum proteins to vary in fish and to indicate the results of an electrophoretic study of the serum proteins in a whitefish, Coregonus clupeaformis, population.

B. Review of Literature

1. History

Nuttall (1904) did the initial work in a field now known as comparative or systematic serology. The basis for his work involved the precipitin reaction whereby the proteins in the serum of one animal are compared to the proteins in the serum of another animal. The degree of serological correspondence, measured by the amount of precipitation of antibodies and antigens in this reaction, is thought to reflect the degree of relationship between the animals. The precipitin reaction occurs when the serum from each of two animals is allowed to react with rabbit antiserum against the proteins in the serum of one of the animals.

Immunological methods make use of a biological system to distinguish among protein relationships which are based upon differences in molecular orientation. This is in contrast to comparison of proteins by electrophoresis where differences in charge, size and shape of the molecules are the basis for evaluation.

The early workers studying fish blood made direct quantitative chemical studies of its protein components with some comparisons of various fishes. Vars (1934), Roche et al. (1940), and Field et al. (1943) did quantitative studies of the components of the blood of various species of fish. Recently, Phillips (1957), Phillips et al. (1958), and Shell (1961) did similar quantitative studies of fishes' blood.

Hall et al. (1926) found that the total nitrogen concentration in the blood of the menhaden, Brevoortia tyrannus, increased with time when the fish were placed under asphyxiating conditions. They later stated that this was not a true increase since the total water in the blood of the menhaden decreased and thus tended to concentrate the protein components. Lepkovsky (1929), however, found that the menhaden under no stress had varying concentrations of total nitrogen in the blood plasma. The albumin-globulin ratio (A/G) varied from 1.28 to 2.08 in the menhaden examined in this study.

Fontaine and Boucher-Firley (1932) found a range of 25 to 70 grams per liter for the total serum proteins with large individual variations, in their comparison of fresh and salt-water species of fish. Demenier (1934) found variability within and among species in his determination of albumin and globulin concentrations. His work on the eel, Anguilla vulgaris, showed a variation for albumin and globulin determinations made during different seasons. Pora (1936) found blood proteins were higher in concentration in the female than the male of a species of cat shark and the opposite results in a

species of skate. He suggested that these apparent sexual differences were due to endocrine influence. Adrianow (1937) showed that the A/G ratio did not change over a period of time in some fishes but in the Crucian carp, Carassius carassius, changes did occur in its A/G ratio. Brachet (1940) in a histochemical study of proteins during embryonal development of some fish, found considerable change in concentration and appearance of different serum protein fractions during ontogeny.

It was not until Tiselius (1937) introduced a quick means of separating proteins by the moving-boundary electrophoretic method that effective comparative serum protein studies could be made. Deutsch and Goodlee (1945) were the first authors to report species-specificity for plasma protein electrophoretic patterns using the Tiselius method. They separated plasma proteins of various animals and found large variations in the distribution of blood proteins among animals. The variations appeared in the form of different mobilities of protein components in an electric field and in the number and relative proportions of the components which did separate in the serum samples. In a later electrophoretic study of the blood serum proteins of some lower animals, Deutsch and McShan (1949) found that species-specificity also existed for electrophoretic protein patterns among these animals. Moore (1945), during the same period, found characteristic species differences in the serum protein electrophoretic patterns, obtained under comparable conditions, for certain mammals, birds and fish. The patterns of each species were reproducible and the differences with respect to quantity and presence or absence of components were significant between species.

2. Species-Specificity and Heredity of Serum Proteins

After 1945, studies were published that substantiated the species-specific nature of serum protein electrophoretic patterns of many species of fish and other animals. (By this time, different electrophoretic methods were in use involving paper, gels and starch as a media for separating serum proteins.) Some of these papers and the animals studied were as follows: Irisawa and Irisawa (1952), sharks; Brandt et al. (1952), birds and their hybrids; Drilhon (1953), eels and carp; Dessauer and Fox (1956, 1958), amphibians and reptiles; Zweig and Crenshaw (1957), Pseudemys turtles; Starr and Fosberg (1957), sharks; Woods and Engle (1957), marine invertebrates and fishes; Planas and Gras (1957), fish; Saito (1957a), fish; Woods et al. (1958), invertebrates; Engle et al. (1958), marine fish; Drilhon (1959), teleosts; Chandrasekhar (1959), cyprinids; Drilhon (1960), teleosts, cyclostomes and selachians; Delcourt (1961), amphibians, reptiles and fish; Das (1961), Indian carp; Gunter et al. (1961), elasmobranchs and teleosts.

The one point lacking in the above papers was whether these species-specific proteins followed a pattern of Mendelian inheritance and thus could definitely be used for the study of phylogenetic relationships. To date, this point has not been completely elucidated. Irwin and Cumley (1940; 1942) by immunogenetic techniques showed conclusively that in pigeon crosses, species-specificity of certain serum proteins fell within the pattern of Mendelian inheritance. They anticipated that all species-specific relationships would be found to be gene determined. Pauling et al. (1949) showed that there

was a difference in electrophoretic mobilities between normal human hemoglobins and those affected by sickle-cell anaemia. Ingram (1957) confirmed this and proved that these differences were under gene influence. The hormone, insulin, was found to differ structurally in five species of mammals in only one portion of the molecule (Sanger and Tuppy, 1951; Brown, Sanger and Kitai, 1955). Smithes (1959) established that beta-globulin fractions C and D, in humans, were genetically controlled by a pair of autosomal alleles with no dominance. All these cases of gene control on protein structure prompted Crick (1958) to indicate that a new field was being developed called protein taxonomy where the amino acid sequences of the proteins of an organism and the comparison of them between species would be studied.

3. Variation in Serum Proteins Under Various Physiological Influences

Drilhon (1954) found concentration and mobility differences in paper electrophoretic patterns of carp by sex. Serum total protein concentration differed (Bailey 1957) between male, non-breeding female and breeding female goldfish after injection of estradiol. Injection of Sockeye salmon with estradiol monobenzoate (Ho and Vanstone, 1961) caused differences between male and female serum protein levels. Serum vitellin was absent from the plasma of male, immature female, and spent female Coho salmon (Vanstone and Ho, 1961). These authors found a lipoprotein (possibly vitellin) in the electrophoretic pattern that was associated with egg formation. Pacific salmon

(Robertson et al., 1961) were found to have sexual differences in total protein, albumin and globulin concentrations while moving from the sea to their spawning sites. In an electrophoretic study on serum proteins, Lecal (1958) placed fish of both sexes in different concentrations of salt water for various periods of time and found percent proportions of components changed and the changes were different for each sex. Electrophoretic examination of the blood of 39 sturgeons showed low A/G ratios for smaller individuals (Magnin, 1960). The author indicated that these differences may be associated with sexual maturity. Drilhon (1954) found differences in electrophoretic patterns of carp serum protein concentration by sex and maturity. Mature and immature stages of elasmobranchs (Saito, 1957d) showed differences in serum total protein concentration and electrophoretic analysis showed a lack of some components in immature forms that mature forms had.

Pacific salmon (Robertson et al., 1961) that underwent starvation for a longer period of time during migration, had decreased concentrations of plasma total proteins, globulins, and albumins. Drilhon et al. (1956) found quantitative differences in albumins and beta-globulins when starved trout were compared with well fed trout. Carp, during hibernation and prolonged fasting, showed a decrease in concentration of serum total proteins, albumins, and beta-globulins (Sorvachev, 1957).

Deutsch and McShan (1949) indicated that in fish and other animals electrophoretic patterns would show serum protein fluctuations due to pathologies. Phillips et al. (1957) found that the

A/G ratio in brown trout affected with kidney disease was the reverse of the value obtained from brown trout not affected by this disease. They suggested that examination of serum proteins in hatchery-reared fish may be an important aid for predicting outbreaks of disease in these fish. Flemming (1958) found serum total protein varied in concentration in blood serum from diseased carp. Sindermann and Mairs (1958) showed that sea herring suffering from acute fungus disease had a sharp reduction in concentration of the albumin fraction of their blood serum. Hyperimmunized progeny of carp had a drastic reduction in total albumin concentration and to a lesser degree in alpha and beta globulins and a sharp rise in gamma globulins (Sorvachev et al., 1962).

Moore (1945) reported that there was evidence of major changes in serum protein fractions of fish and other animals with age and development. In a study of Coho salmon (Vanstone and Ho, 1961) plasma proteins, one protein fraction increased with age while in smolt transformation another protein fraction disappeared.

Deutsch and McShan (1949) noted that, in general, differences between species electrophoretic serum patterns of fish were greater than between any two individuals of one species. Sindermann and Mairs (1961), however, found marked individual variations in serum total protein concentration in both pre- and post-spawning alewives. They found no major changes in paper electrophoretic patterns of alewives due to reproduction or freshwater migration. A study of sturgeon plasma proteins (Magnin, 1958) showed wide individual variations in concentrations especially in the albumin fraction.

Bendett et al. (1941) found that a dilution of blood serum occurs in migrating Atlantic salmon. This would account for a decrease in serum total protein concentration which these authors did not mention in this study. Robertson et al. (1961), however, found definite concentration changes in albumin and globulin fractions of migrating Pacific salmon. Drilhon et al. (1958) found that during the freshwater migration of two species of trout, there was no effect on the serum protein electrophoretic patterns.

Fish placed under different osmotic pressures showed altered serum total protein, albumin, and globulin concentrations (Cordier and Barnoud, 1959).

Meisner and Hickman (1962) were able to show alterations in the A/G ratio of rainbow trout placed under two temperatures and no sign of effect on the serum total protein pattern due to exposure to two different photoperiods.

Seasonal variations (Saito, 1957c) in total serum protein and albumin concentrations were found in mackeral and carp. Also, Flemming (1958) noted that the A/G ratios varied in overwintered carp.

Electrophoretic serum protein patterns in fish, exposed to polluted waters and waters with various concentrations of chemical agents, (Fujiya, 1961) showed concentration shifts in the various components making up the patterns.

4. Other Protein Systems and Electrophoretic Methods for Detecting Phylogenetic Relationships

Bier (1959), Wolstenholme and Millar (1956), and Block et al. (1958) discuss the theory and general methods of different

electrophoretic techniques. Grabar and Williams (1955) have described an immuno-electrophoretic method for separating antigenic substances through the combination of techniques used in serology and electrophoresis. Each constituent of the protein mixture studied gave independent specific precipitin bands, which could be distinguished owing to their immunological specificity and defined by their relative electrophoretic mobility. However, no means of making quantitative measurements has been applied to this method. Bargetzi (1958) separated two forms of whitefish by using this method on the basis of patterns of different antigens.

Hashimoto and Matsuura (1958-59) have discovered multiple hemoglobins in five species of fish by electrophoretic analysis. Connell (1953), using the Tiselius method of electrophoresis on fish muscle protein extracts, has found differences in the patterns such that he could separate the different species of fish.

METHODS AND MATERIALS

A group of whitefish, Coregonus clupeaformis, in Munising Bay, (Michigan) Lake Superior was chosen for study because some of them exhibited an unusually slow growth rate (Edsall, 1960) whereas others of the group grew at a normal rate. It was thought that any genetic basis for these different growth rates would become apparent through morphometric and blood studies.

A field kit (rectangular wooden box) was constructed such that the following materials could be carried aboard a commercial fishing boat used in this study:

1. 75 centrifuge tubes and four racks for holding blood samples.
2. 12 number 20 gauge needles with 2 cc. syringes for taking blood.
3. Tags to identify the fish in order to relate blood sample with scale samples to be taken on shore.
4. Various waterproof marking pencils.
5. Tissues for wiping needles.
6. 0.75% isotonic (fish blood) saline solution for washing the needles and syringes.

The blood samples were taken on July 19-20, 1961 during the hours of 6 A.M. and 10 A.M. Approximately 1 to 2 cc. of blood were removed from each fish by cardiac puncture with hypodermic needles and placed in the centrifuge tubes to clot. Clotting occurred within approximately one minute. No sex separation of the fish was made. On shore the blood samples were centrifuged for 15 minutes at 3500 rpm to separate the serum from the cells and fibrinogen. The serum samples were then placed in five cc. test tubes covered with a plastic film (Dow parafilm). The serum samples were frozen at a local freezer plant and later transported to the laboratory under dry-ice protection from thawing. All of the samples were analyzed within a period of 5 to 12 days after being taken.

The 52 fish sampled ranged in age from 2 to 11 years. Their size and weight ranged from 24 to 47 cm. and 7 to 57 oz. The ages of the fish were determined from scale annuli by an experienced worker (Clary, 1962) who had previously aged 1400 fish from this population. Each fish was aged four times.

The analytical work was done on the Spinco-Beckman Model-R paper electrophoresis system which consisted of a standard Durrum cell, a constant current power supply ("Duostat") and associated equipment for dyeing, rinsing, and fixing the paper strips. The buffer used in this study was the Spinco B-2 consisting of a mixture of diethyl barbituric acid and sodium diethyl barbiturate to provide a liter of solution with a pH of 8.6 and an ionic strength of 0.075. All separations were made at a constant current of five milliamperes per cell during a period of 16 hours at room temperature. Each cell contained eight strips, 30 mm. in width.

Ten microliters of serum were placed on each of eight strips. Following the 16-hour separation, the strips were dried in an oven at 120° to 130° C., dyed in bromphenol blue (30 minutes), then rinsed, fixed and dried according to the procedure specified by Beckman Instruments, Inc. (Spinco Procedure B). The resulting dyed strips were analyzed by the Spinco Analytrol (Model RB), which translates the protein components on the strips into a pen-drawn curve, with the height of the curve being in direct proportion to the protein density at any given point on the paper strips. This apparatus provides an automatic integration of the area subtended by this curve, thus allowing calculation of relative protein densities for any portion of the profile. At pH 8.6, most of the blood serum protein exhibited anodal migration.

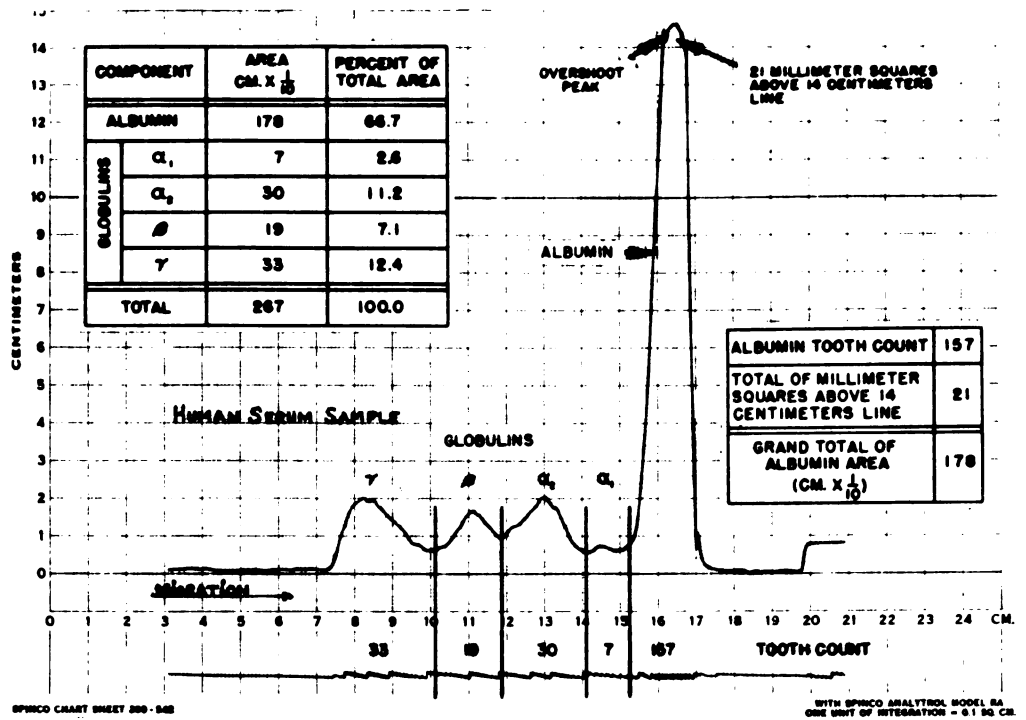
Under these existing standardized conditions, identical proteins will occur at the same location on the paper strips and differences among paper strips must be interpreted as resulting from the presence

or absence of different proteins or from differences in concentrations of the same proteins. Sixteen 10 microliter applications from one individual fish serum sample were placed on 16 paper strips and analyzed at one time. The results indicated that approximately a three percent error in reproducibility could be expected between pattern percentage composition values for the protein components separated.

Each protein component was given a number starting with number one for the slowest moving component to number five, the fastest moving component (Fig. 1). An analysis of variance on a one-way classification was done to determine if there was a significant difference amongst age-groups for percentage composition of each protein component separated.

The percentage composition for each protein component which separated was calculated from the data on the analytrol sheets (Table 1). For comparative purposes the percentage data was transformed using the arcsin method suggested by Snedecor (1956). This transformation was necessary since the data exhibited non-additivity of effects and heterogeneity of variance which would not satisfy the requirements of an analysis of variances (requires additivity of effects and homogeneity of variance). After the data were transformed, Tukey's test of additivity of effects and Bartlett's test of homogeneity of variance were applied (Snedecor, 1956) and the data indicated, respectively, additivity of effects and homogeneity of variance. Keul's test (Snedecor, 1956) for comparison of means was

**Figure 1. Analytrol Sheets showing a Human
and two Whitefish Serum Protein
Electrophoretic Patterns.**



EVALUATION OF ANALYTROL TRACE

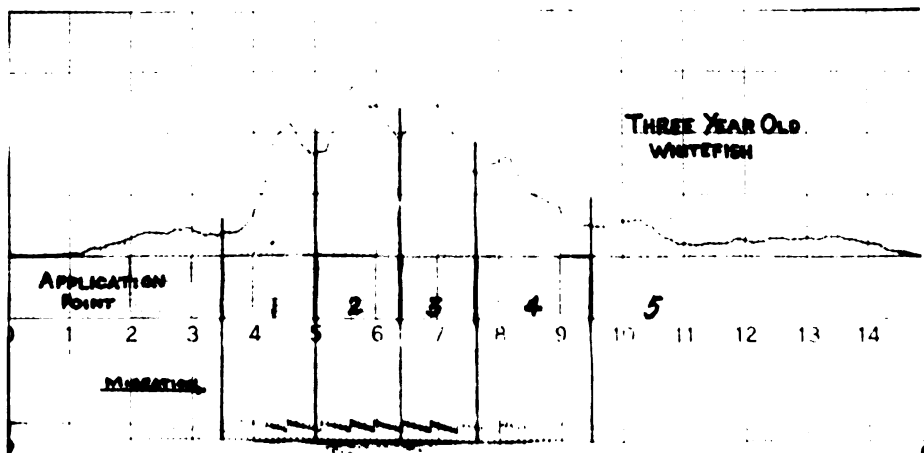
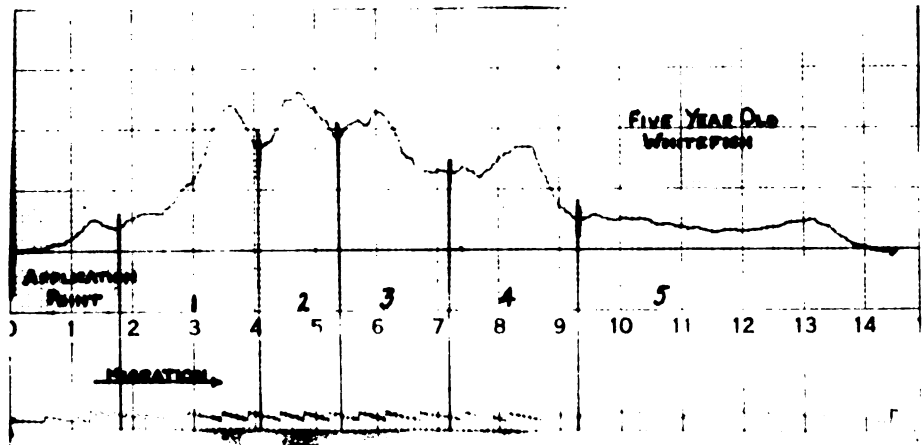


TABLE 1. Percentage Composition of Serum Protein Components

Component		1	2	3	4	5
Age Group	Sample					
III	1	15.22	21.72	13.74	26.70	16.00
	24	25.06	21.37	17.69	18.24	14.92
	25	16.82	23.87	18.48	23.94	13.10
	59	17.52	24.23	23.40	17.41	13.03
IV	2	17.32	21.26	14.96	22.60	20.71
	17	17.73	26.60	12.03	33.57	7.60
	30	18.92	18.13	27.60	15.98	16.61
	31	15.05	24.01	24.87	20.72	12.07
	33	19.01	16.64	21.31	28.94	9.51
	35	20.40	21.48	16.11	29.53	7.51
	36	16.13	23.19	16.64	29.24	12.60
	42	16.69	17.04	17.97	23.36	21.08
	43	28.32	16.36	16.65	19.51	16.89
	52	38.34	17.15	12.61	18.26	7.46
	53	16.63	22.46	19.13	26.62	12.47
	61	15.82	26.89	17.40	24.20	9.01
	71	24.04	22.16	14.62	22.78	7.49
	73	19.44	17.82	33.22	9.72	17.01
	77	20.23	19.67	18.97	26.42	12.22
	79	35.87	13.70	16.51	15.36	9.85
	81	35.12	27.44	11.84	14.51	6.91
	82	25.68	17.65	23.27	19.74	10.75
	90	19.74	22.85	29.42	17.99	7.31
	91	18.02	18.02	18.50	23.38	19.53
	92	16.41	18.41	18.41	27.62	14.73

TABLE 1. (cont.)

Component	1	2	3	4	5
Age Group IV (cont.)					
Sample					
93	21.00	21.82	20.73	24.98	9.38
94	18.05	22.99	25.52	15.73	15.46
95	21.00	18.08	21.00	28.41	9.50
97	20.77	21.71	16.05	20.77	17.28
100	34.69	22.37	10.40	8.74	20.36
110	22.26	17.12	18.83	20.54	19.06
111	25.14	14.85	21.14	12.00	24.57
120	25.08	17.44	19.08	17.44	17.44
122	32.59	16.82	21.03	18.92	7.36
V					
16	18.15	24.88	14.79	20.51	17.48
44	24.16	20.62	16.73	17.56	16.12
47	20.87	20.93	16.37	24.36	14.13
48	32.57	17.06	12.22	15.18	12.86
56	27.66	20.77	13.63	25.50	9.28
58	27.09	15.05	14.30	22.57	16.55
62	19.95	19.23	16.43	17.49	7.56
63	21.24	23.36	18.41	25.58	8.00
64	29.72	22.15	11.45	23.18	7.78
65	33.29	16.52	17.41	20.56	8.68
68	21.42	23.25	17.44	18.68	16.19
69	19.95	19.79	19.83	25.53	12.16
85	36.47	19.85	12.92	9.23	18.00
96	20.22	18.66	19.91	27.62	11.07
118	38.07	22.47	10.86	13.48	12.48
IX					
70	31.78	15.55	21.60	15.71	12.52
72	31.45	17.92	18.47	23.09	6.38

applied for those age-group components which showed significant differences in their means (Table 2). There were enough fish sampled in age-groups III, IV, V and IX to allow for comparison of similar protein components in these age-groups.

TABLE 2. Keul's Test of Least Significant Difference among Arcsin Transformed Component Means ^a

Component one				
Age Group	Mean	Mean Minus 25.49	Mean Minus 28.17	Mean Minus 30.37
IX	34.22	8.73 (4.37)	6.05 (3.98)	3.85 (3.31)
V	30.37	4.88 (3.98)	2.20 <u>(3.31)^b</u>	
IV	28.17	2.68 <u>(3.31)</u>		
III	25.49			
Component three				
Age Group	Mean	Mean Minus 23.20	Mean Minus 25.26	Mean Minus 25.80
IX	26.58	3.38 (3.09)	1.32 (2.81)	0.78 (2.34)
IV	25.80	2.60 (2.81)	0.54 (2.34)	
III	25.26	2.06 <u>(2.34)</u>		
V	23.20			

^a Refer to Snedecor (1956) p. 253 for explanation.

^b The underlined numbers in the tables indicate means that are not significantly different from those listed in the left-hand column of the table.

RESULTS

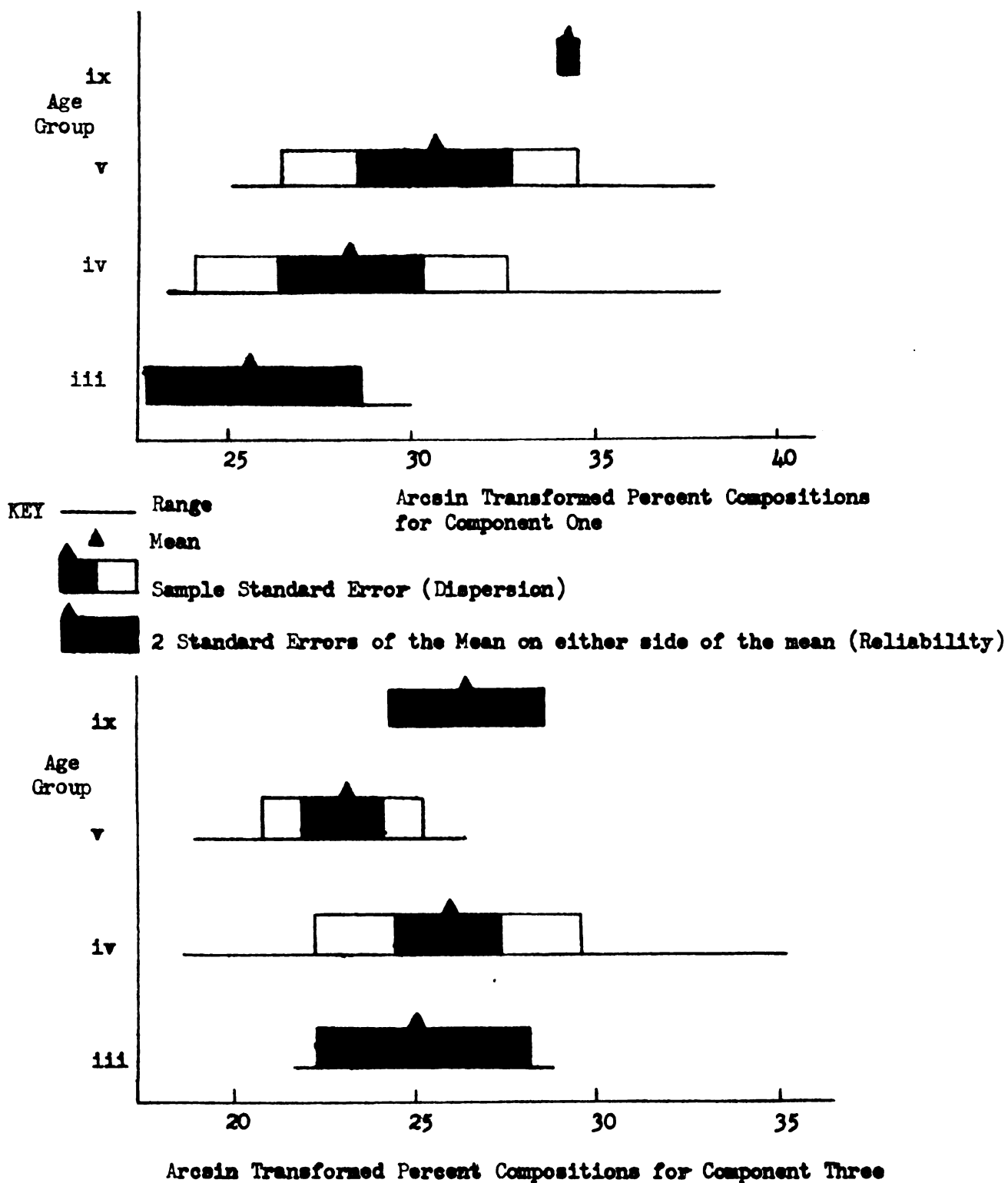
The mode of the electrophoretic patterns and the number of serum protein components separated remained the same for all of the age-groups that were sampled. A comparison of all the paper strips and their particular patterns from this group of fish showed the same serum protein components appearing upon each separation. The percentage composition of components one and three showed a significant difference among age groups at the 95 percent confidence level. These components were the slower moving ones and would be in the globulin portion of the serum if compared with human sera protein patterns.

Age-group IX had a greater percentage composition for component one than age-groups III, IV, and V (Table 3 and Fig. 2). Percentage composition for age-group V was greater than that of age-group III but the same as that in age-group IV. Age-group IV had the same percentage composition as age-group III for component one.

TABLE 3. Arcsin Transformed Component Statistics

Component one				
Age-group	III	IV	V	IX
Mean	25.49	28.17	30.37	34.22
Standard Deviation	3.12	4.37	4.14	0.17
Number in Sample	4	30	16	2
Component three				
Age-group	III	IV	V	IX
Mean	25.26	25.80	23.20	26.58
Standard Deviation	2.91	3.70	2.25	1.52
Number in Sample	4	30	16	2

Figure 2. Graphical Representation of Statistics for Serum Protein Components One and Three



Age-group IX had a greater percentage composition for component three than age-group V but the same percentage composition as age-groups III and IV (Table 3 and Fig. 2). Percentage composition for age-group IV was the same as age-groups III and V. Age-group III had the same percentage composition for component three as age-group V.

DISCUSSION AND CONCLUSIONS

Protein component one was the slowest moving fraction and was in the globulin portion of the electrophoretic pattern. The increase of this globulin fraction with fish age may be related to increases in globulin antibody production as the fish is exposed to various diseases during life or it may be a normal development as the fish matures. Similar results have been obtained for globulins in mammals (Moore et al., 1945), in birds (Brandt et al., 1951), and in fish (Vanstone and Ho, 1961).

Protein component three increased in concentration with age of the fish but at age V there was a decrease in concentration of this component. No apparent reason could be found for this change. This protein component is also a globulin and the physiological stage of the fish at age V may require an increased use of this component accompanied by a slow replacement. It is possible that the number of fish sampled for this age-group may not have been large enough to provide an unbiased evaluation of this component's concentration.

The blood tissue is perhaps more dependent on the physiological state of an organism as a whole, than is any other tissue. The

author has cited studies whereby sex, spawning, food, osmotic pressure, disease, temperature, light, age, hibernation, hormones, oxygen depletion and season have had some effect on the total serum protein complement of a fish. All of these conditions are interrelated and no one case is independent in its action. There is a close correlation between the blood, the protein system and the physiological state of the fish rather than to any one particular condition. Much of the variation in the serum proteins caused by different conditions places a stress on the fish that is reacted to by the fish, each time, in the same manner. There is an increase in globulin with a decrease in albumin concentration. Serum protein variations in the blood of a fish can be compared to a glass full of water. The water can be poured out of the glass but the water remaining in the glass still retains its same molecular structure. If workers are to use electrophoretic patterns of the blood serum proteins of fish for phylogenetic studies, they should be aware of the variables causing changes in these patterns. Concentrations of serum proteins may change, but this will not affect the molecular structure or species-specific nature of these proteins.

Gross changes in electrophoretic patterns are the results of changes in the concentration of protein components and the addition or subtraction of a component. Many of the papers reviewed in this study have stated that a system of standard conditions is required to allow for comparison of protein patterns among animals. Saito (1957b) has shown that re-use of buffers can change electrophoretic

patterns and Kohn (1958) has found that large quantities of serum protein can mask minor components in electrophoretic separations. The electrophoretic method which allows for careful characterization of each curve with respect to shape, symmetry, asymmetry, width of each protein peak, and of the tendency of each adjacent peak to separate from or approach each other, will provide the better basis for comparison among serum proteins of fish.

Correct descriptions of serum protein patterns should be described under the headings of mobility with defined conditions of buffer ions, ionic strength, pH, and protein concentration. To obtain reliable quantitative electrophoretic measurements of fish sera the number of fish sampled must be large, the fish healthy and separated by age and sex.

Whitefish are well-known for their complicated morphological variations. No agreement among the systematists on the interpretation of their variations has been reached. The information gained from serum protein comparisons enables a better elucidation of the taxonomic positions of these fish.

Svårdson (1948) has started a genetic study of various populations of whitefish as opposed to the traditional morphological approaches in separating these fish. The more direct the examination of gene action on the determination of the structure of the animal, the easier will be the determination of its taxonomic position.

The biological concept of individual, species, and genus reflects different orders of organization which are manifested by

morphological features. It is not unlikely, that within the complexity of the protein molecule, these same levels of organization will be uncovered. If this occurs, the biologist will be called on to work with the biochemist to elaborate the species concept.

SUMMARY

The conditions, studied by other authors, causing variation in total serum protein levels in fish and measured by chemical and electrophoretic methods are reviewed. Sex, spawning, food, osmotic pressure, disease, temperature, light, age, hibernation, hormones, oxygen depletion and season have some effect on either changing concentration, decreasing or increasing a particular component and taking a component away or adding one to the total serum protein complement of a fish.

A study of the serum protein electrophoretic patterns of a whitefish population, Coregonus clupeaformis, revealed significant increases with age of two serum globulin components.

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