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A COMPARATIVE STUDY OF THE PLASMA PROTEINS OF SOME TRANSVAAL FRESHWATER FISH

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Johannesburg, 1973

I hereby declare that this thesis entitled: A Comparative Study of the Plasma Protains of some Transvaal Freshwater Fish, is my own work, and that the work forming the basis of the thesis has not been incorporated in a thesis submitted for another degree by myself.

Signed:.....

Date:.....14/4/73.....

To Rie and Karen.

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ABSTRACT

The plasma proteins of *L. umbratus*, *L. capensis*, *C. gariepinus* and *B. holubi* have been investigated by means of cellulose-acetate and polyacrylamide gel electrophoresis. Characteristic patterns were obtained for each species although variations occur within a certain basic framework. Possible factors which influence these patterns are discussed.

Certain of the fractions of *L. umbratus*, *L. capensis* and *B. holubi* were isolated by means of chromatography on Sephadex G-100, preparative polyacrylamide gel electrophoresis and isoelectric focusing. Approximate average molecular weights of these fractions were found to vary between 55,000 and above 120,000 and isoelectric points between 5,81 and 3,8. Certain fractions were found to exist in dimeric form.

It is concluded that the application of the human nomenclature to fish plasma protein fractions is of dubious value and that the fish fractions can only be classified after thorough biochemical investigation.

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1. INTRODUCTION

The plasma proteins comprise a dynamic system with varied functions, the components of which are variously classified according to the mode of detection. Putnam (1965) maintains that they are correctly considered as a whole because of their similar biosynthetic origin, their participation in common processes, and their occurence together as the major extracellular components of the circulatory system. The plasma proteins occuring in different species may or may not serve the same physiological functions and the terminology of the one may or may not be applicable to the other. Very little is known about the lower vertebrate and invertebrate proteins occuring in the bodyfluids (Engle and Woods, 1960) and most, if not all, of our knowledge has come from the human and higher vertebrates. From a comparative point of view, a closer study of the plasma proteins of the lower animals could yield valuable information concerning the systematic classifications and phylogenetic relationships of animals and at the same time a closer study could be made of the comparative biochemistry of the plasma proteins.

Information concering the plasma proteins of fish is fragmentary and scattered and some of the data available in the literature were derived when analytical methods were less reliable (Satchell, 1971). The total plasma protein is very variable ranging from 1,68 g% in Cynascion arenarius to 6,19 g% in Sciaenops ocellata (Sulya et al, 1960). This latter figure approached the 7 g% of human blood. The ray, Raja kenojei, and the shark, Heterodontus japonicus lack serum albumin (Irisawa and Irisawa, 1954) and the suggestion has been made that all elasmobranch fish lack this

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protein. (Sulya et al, 1961). Albumin is apparently present in the blood of advanced teleosts and Field et al (1943) reported that the total plasma protein of carp was 4,5 g% and that albumin accounted for 2,82 g%. A further aspect of the plasma protein system of fish has been stressed by Klontz et al (1965). They separated the serum proteins of Salmo gairdneri electrophoretically and recognised albumin, alpha$_1$, alpha$_2$, beta$_1$, beta$_2$, and gamma globulins but noted that none of these six can be correlated directly with any of the mammalian serum proteins. In the same study, inoculation of the fish with Sacramento River chinook disease virus caused an elevation of the beta$_2$ globins coincident with marked lymphoid hyperplasia. This suggests that in fish antibody protein is associated with beta$_2$ rather than with the gamma globulin fraction as it is in mammals (Klontz et al, 1965).

From the foregoing it is evident that much remains to be learnt about the general biochemistry and physiology of fish plasma proteins (see also p5 and review by Booke, 1954). Concerning the plasma protein system of the South African freshwater fishes, virtually nothing is known. The physiological chemistry of local fish blood if of importance in the study of the overall biology of these fish seeing that attempts are to be made to breed them for commercial purposes. (Hattingh and Du Toit, 1973). A further aspect is that some of the mudfish species are becomming a threat to the existence of other more popular sport fish (such as yellow-fish) due to overproduction and therefore a closer study of their biology is necessary for cropping and conservation procedures.

The freshwater bony fish (Teleostei) occuring in Transvaal are vast in number, and some 74 species are known (Le Roux and Steyn, 1968). Of these the common mudfish species, the yellow-fish species, the

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catfish or barbel species and the bream species are by far the most abundant and popular (for food). As has been said, little is known about the physiology and biochemistry of these fish (see Hattingh, 1972) and the experimental approach in this thesis to the study of the plasma proteins of some of them, has been as follows:-

1) Of the available indigenous species, it was decided to study the common mudsuckers or mudfish, Labeo umbratus (Smith 1841) and Labeo capensis (Smith, 1841) because of their abundance. These fish produce at such a rate that the existence of Barbus holubi (Steindachner, 1894) or yellow-fish is threatened. All three fish occur in the same waters, but the yellow-fish is the more popular and is also very sporting for the fisherman, whereas the mud-suckers are rarely caught by hook and line. The biology of the yellowfish is therefore also of interest and, in addition, the barbel or Clarias gariepinus (Burchell, 1822) was also investigated. This fish is fairly abundant and provides a good high protein food. Taken together, these four fish species represent the major inhabitants of Transvaal rivers and lakes.

2) Blood was obtained from representatives of all four species and the plasma protein system analysed by means of cellulose-acetate and polyacrylamide gel electrophoresis. It was hoped that some sort of "normal" or "standard" pattern would result for each species. The different electro-phoretograms were also investigated for the influence if any, of sex, reproductive activity, locality, etc. and finally these patterns were compared with each other and with that of the human.

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3) From the observed patterns, one or two major bands present in all cases were chosen, isolated by electrophoretic and other techniques and then studied (average molecular weight, isoelectric point, absorption spectrum, etc.) The results of the various species were then compared with each other.

This study then, was an effort to elucidate the basic plasma protein pattern of the four species involved and an attempt at the isolation and purification of one or two major components. It shall be shown that the basic pattern is very variable in all four cases but that certain bands are always present. On the comparative side it shall be shown that the two mudfish species have very similar patterns but that the yellowfish and barbel differ markedly. None of the patterns obtained strictly corresponded to that of the human.

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2. REVIEW OF LITERATURE

(Concluded May, 1973).

An excellent review concerning fish proteins as a whole has been published by Hamoir (1955). Booke (1964) and Hunn (1967) have published reviews on fish blood and plasma proteins in general. The present review will mostly be concerned with reports on the Teleosti.

2.1 The plasma proteins:

The study of fish blood proteins commenced when Lepkovsky (1930) used Howe's (1923) methods for the fractionation of serum proteins. He quoted the albumin: globulin ratios for the goosefish and dogfish to be 1,20 to 2,08 and 0,23 to 0,39 respectively. No electrophoretic studies were carried out. Field et al (1943) reported that the total plasma protein of carp was 4,15g%; of this albumin accounted for 2,82 g, globulin 0,79 g and fibrinogen 0,23 g. In 1945 Deutsch and Goodloe studied the blood proteins of the carp with a modified Tiselius apparatus and identified gamma, delta, beta and alpha globulins and albumin. The latter was found to consist of two parts. A year later, Deutsch and McShan (1949) extented these observations and reported 4-12 components on electrophoretic separation. Morris (1959) examined the blood of Australian freshwater fish by filter paper electrophoresis and found that the lipoproteins had a high electrophoretic mobility and were integrated with the globins or the albumins. Beuker (1961) found the catfish to have 5 fractions of which the gamma globulins comprised 37% and the albumins 10%. A summary of other findings up to 1961 is given in table 1 (p_{11} to $_{13}$).

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Engle and Woods (1960) also summarized available knowledge on the separation of proteins by different electrophoretic techiniques in fish and reported the following :

boundary, 4-12 components; filter paper, 4-6 components; agar 5-6 components and starch gel, 4-11 components. Neither isolation nor characterization had been performed up to that time (1961) and the nomenclature was based on comparisons with the human electrophoretogram or according to various crude fractionation techniques. (Putnam, 1960). (see p92 for a summary of patterns obtained by various workers).

2.2 The taxonomic value of plasma proteins:

The belief that electrophoretic patterns of plasma proteins may provide information of taxonomic value has led to many publications. Deutsch and McShan (1949) report 12 separate components in the electrophoretogram of the plasma of Perca flavescens. Mairs and Sinderman (1960) published observations on the herring and concluded that it is inadvisable to assume absolute specificity of electrophoresis patterns in closely related groups unless considerable numbers are ... examined. Bauker (1961) found catfish serum proteins to differ markedly from that of higher vertebrates; gamma 37%, $beta_1$ 13%, $beta_2$ 19%, alpha 21% and albumin 10%. Drilhon et al (1961) used agar, paper and starch gel electrophoresis on pike sera, found from 5 to 8 fractions and remarked on the absence of a fraction comparable in mobility to human $gamma_2$ globulin but noted the abundance of the $gamma_1$. Engle and Woods (1960) also

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stated that fractions with mobility of human gamma globulin are either absent or present in only small amounts in most teleosts. The work of Drilhon (195[illegible], O'Rourke [illegible] (1960) and Sanders (1964) provided much information about the patterns of bass, trout, carp, catfish, perch and whitefish. The patterns for any one species were fairly constant. Mairs and Sinderman (1960) found geographical differences in the serum patterns of Clupea harengus and Nyman (1965) showed that the serum proteins of two hundred specimens of Salmo salar were remarkably constant among themselves. These patterns, however, differed from those of the same species at a different geographical locality. (see also Tsuyuki et al, 1966).

In 1967, Thompson suggested a standard method for the routine identification of fish species by means of polyacrylamide electrophoresis and Tsuyuki and Roberts (1965) found that when a hybird species had beed reared, no new protein bands were formed, but the hybrid gives electrophoretic characteristics of one of the parental types (Sanders, 1964, Uthe et al, 1966). Similar attempts at classification has been applied to the serum transferrins by Greysel et al (1966) and Barrett and Tsuyuki (1967).

Snieszko et al (1966) found that a fraction in rainbow trout serum which was classed as a globulin because of its comparable mobility with human alpha globulin, was in fact classified as an albumin by chemical analysis. The application of human serum electrophoretic fractions (nomenclature) to fish sera therefore appears to be of doubtful value and may in fact serve to perpetuate

a confusion (Mulcahy, 1970). Several other studies along the same lines have been published in an effort to obtain "standards" (Badawi, 1971; Mulcahy, 1970; Hattingh, 1972; and Mills and Taylour, 1971). All these studies, however, lack any definite chemical analysis and there is thus need for the study of the molecular and biological properties of these fractions in fishes.

2.3 Environmental and other influences on the plasma proteins:

Season is known to influence the total amount of protein in the plasma. Robinson et al (1961) showed that in Oncorhynchus tschawytscha migrating activity lowers the protein value e.g. in the sea these animals have 6,6 g% protein and during the spring spawing run it drops to 1.7 g%. Similar observations have been made by Urist and Van de Putte (1967). Saito (1957) previously found that in the mackerel and carp the concentrations of total protein and albumin were lower in winter time than in the warm time. In the same study, he also showed that the albumin fraction was lower in cultured mackerel that in natural ones.

In 1967, Summerfelt et al showed that there is no significant difference between the plasma protein levels of male and female goldfish. They also found that metabolic factors influence the protein level and this agrees with the work of Shell (1961). Sorvachev et al (1962), Thomas and McCrimmon (1964), Booke (1965) and Sano (1960) all found variations with age and showed that the alpha and beta globulins increase gradually with increasing age. Thomas and McCrimmon (1964) also showed variations with state of life history and sex.

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This differs from the results of Summerfelt et al (1967) but corresponds to the work of Sano (1960). The latter showed that injection of Oncorhynchus nerka with oestradiol monobenzoate causes differences between male and female serum protein levels.

Lysak and Wojcik (1960), Drilhon (1954) and Cordier et al (1957) studied the influence of diet and the latter showed a marked reduction in protein levels with starvation (see also Fujuya 1961; Sorvachev, 1957; Khailov, 1962; and Thomas and McCrimmon, 1964). The changes observed show that certain fractions are utilized preferentially. Sorvachev (1957) found the decrease to be at the expense of the albumins and later at the expense of the alpha and beta globulins. The gamma globulins showed an increase in absolute values (Khailov, 1962) and this suggests that either blood volume decreased or that gamma golbulin entered the blood stream as a result of protein breakdown in other tissues (Love, 1970).

Studies of the influence of disease on the levels have been made by Thomas and McCrimmon (1964), Fujiya (1961), Mulcahy (1971) and Klontz et al (1965) who all noted variations in various fractions, but seeing that no exact knowledge about these fractions was available nothing more then speculation concerning the proteins involved could be made.

Various other studies have been performed. Lecal (1958) studied the effect of the salinity factor on fish blood and Meisner and Hickman (1962) showed that temperature influences

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the A/G ratio in trout and salmon and that photoperiod had no significant effect. Bouch and Ball (1965) studied the influence of an oxygen pulse on the serum proteins and Kenyon (1967) showed that removal of the liver was followed by a 25% fall in the concentration of plasma proteins and a 65% rise in the concentration of plasma amino acids.

Finally, special mention must be made of further work by Bouch and Ball (1966). They showed that the method of capture has a profound effect on the plasma protein value, usually decreasing it and an effect on the various fractions obtained on electrophoresis was also observed; some decreasing and others increasing. This important finding is discussed on p 41 .

Table 1, which is by no means exhaustive, summarizes most of the relavent findings on the plasma proteins of the Teleosti up to date.

From the foregoing it is evident that much remains to be learnt about fish blood. Many electrophoretic and other analysis have been performed and a fair amount of knowledge of the values of the plasma proteins and the factors influencing them have been obtained. The nomenclature used is usually based on that of the human pattern and we do not really know whether this is applicable to the fish proteins (see p 92 for various patterns). More definite analysis concerning the nature and chemical properties of these proteins is necessary before this system and the variations observed can really be understood.

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TABLE 1

Summary of available results of the electrophoresis and value of plasma proteins from the Teleosti

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Species	Fluid analysed	Mean Protein concentration g%	Methods	Number of fractions	Remarks	Reference
Menhaden	P	1,57	Kjeldahl	-	Low A/G ration	Lepkovsky, 1930
Goosefish	P	1,82		-	Low A/G ratio	
Trout	P	3,46	Colorimetric	-	-	Field et al, 1943
Carp	P	4,15		3		
Rainbow Trout	S	5,1	Kjeldahl Tiselius	4-12	Relative high A/G ratio	Deutsch and McShan, 1949
Lake Trout	S	5,0				
German brown Trout	S	4,0				
Whitefish	S	5,8			A/G ratio; 2,1	
Lake Herring	S	5,0				
Carp	S	3,2				
Channal Catfish	S	4,3				
Eel Catfish	S	4,5				
White Catfish	S	3,3				
Bullhead	S	3,8				
Sucker	S	5,1				
Buffalo fish	S	4,5				
Trout	P	-	Filter paper electrophoresis	4	Lipoprotein migrates with a globulin or albumin	Morris, 1959
Catfish	S	-	Paper electrophoresis	5	gamma globulin 37%, albumin 10%	Reuker, 1961

Species	Fluid analysed	Mean Protein concentration %	Methods	Number of fractions	Remarks	Reference
Salmon	P	6,6-1,7	-	-	-	Robertson et al, 1961
Carp	S	-	Tiselius	6		Deutsch and Keonig, 1956
Catfish	S	-		8		
Perk	S	-		10		
Pike	S	-		7		
Trout	S	-		8		
Whitefish	S	-		10		
Chinook Salmon	S	7,57	Nessler	-	-	Favour, 1958
Whitefish	P	4,19	Gravimetric	-	-	Robertson, 1954
Bleak	S	4,39	Kjeldahl	-	-	Lustig and Ernst, 1937
Carp	S	5,0		-	-	
Berbel	S	3,69		-	-	
Barracuda	S	3,3	Refractometry	-	-	Becker et al, 1958
Spanish Mackeral	S	6,5		-	-	
Grouper	S	2,8		-	-	
Pike	S	-	Agar, paper and starch electrophoresis	5-8	γ1-globulin abundant. γ2 globulin absent.	Drilhon et al, 1961
Salmon	S	-	Starch gel	5	Geographical variations	Nyman, 1965
Tilapia	S	-	Paper electrophoresis	6-7	-	Badawi, 1971

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