PLASMA PROTEINS OF *BARBUS HOLUBI* AND *CLARIAS GARIEPINUS* (TELEOSTEI)

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ABSTRACT

The plasma proteins of the teleosts, *Barbus holubi* and *Clarias gariepinus* were investigated by means of cellulose-acetate and polyacrylamide gel electrophoresis. Characteristic patterns were obtained with both methods for each species. It was found that the application of human nomenclature to the patterns obtained in the fish species studied is not valid. Fractions 12 and 17 of *B. holubi* were further isolated and it was found that the approximate average molecular mass of fraction 12 is 55 500 and the isoelectric point 5,81. In the case of fraction 17 the values were found to be 89 100 and about 3,8 respectively.

INTRODUCTION

Information concerning the biochemistry of fish plasma proteins in general is fragmentary and most studies have only employed electrophoresis (Satchell 1971). Booke (1964) has reviewed the general patterns of these plasma proteins. The plasma protein electrophoretograms obtained in these studies have usually been compared to that of man and corresponding fractions are then named according to the human nomenclature (Klontz *et al.* 1965). Mulcahy (1970), Klontz *et al.* (1965) and Perrier *et al* (1973) have all pointed out that this procedure is not satisfactory and might in fact cause a great amount of confusion. The biochemical nature of fish plasma proteins therefore merits further study.

Equally little information is available on the plasma proteins of South African freshwater fish (Hattingh 1972). A closer study of these proteins in two common species, *Barbus holubi* (Steindacher), the yellowfish, and *Clarias gariepinus* (Burchell), the barbel, was therefore undertaken.

MATERIALS AND METHODS

The fish used in this study were all healthy and mature. The *C. gariepinus* specimens were obtained from the Lowveld Provincial Fisheries Department and the *B. holubi* specimens from the Vaal River. The methods used for anaesthetization, blood sampling, centrifugation, plasma protein determination, etc., have been described previously (Hattingh 1972).

The methods used for cellulose-acetate and polyacrylamide gel electrophoresis (5% gels) have been described in detail (Hattingh 1972).

Certain fractions from *B. holubi* were isolated and studied. Initially, salt precipitation with Na_2SO_4 was carried out for 3 hours at 37°C, using salt concentrations of 10%; 14%; 18%; 22% and 26% according to the method of Howe (1923). The supernatant fluids were subsequently examined by gel electrophoresis. Control experiments showed that the 3-hour exposure as such had no effect on the electrophoretogram.

Secondly, 3 ml samples were precipitated with 26% Na_2SO_4 and centrifuged for 30 minutes after the 3-hour exposure at 30 000 g in a Sorvall refrigerated centrifuge. The supernatant liquors were then subjected to the following separation procedures:

(*a*) Chromatography on Sephadex G-100 equilibrated with Tris-HCl buffer, pH 7,5 and 0,05 M. The column size used was 2,5 × 93 cm. Elution was carried out at 0°C at 12–15 ml/h and was recorded with a LKB Uvicord recorder at 254 nm (2 ml fractions collected). Absorbance of all fractions was also recorded in a Perkin-Elmer spectrophotometer at 260 and 280 nm. Twenty-five microlitre of the fractions obtained was run on 5% polyacrylamide gels for analytical purposes. The same columns were used for approximate average molecular mass determinations according to the method of Andrews (1965).

(*b*) Isoelectric focusing carried out in a LKB 8010 electrofocusing column using a linear 0–50% sucrose density gradient and LKB ampholine of pH range 5–8 at a concentration of 1%. The column was maintained at 0°C and electrofocusing was carried out for 72 hours at a potential of 700 V, and pH was measured with a Radiometer model 25 pH meter to an accuracy of 0,02 pH units.

After separation and visualization of the fractions, the aliquots, containing the desired proteins, were pooled and dialysed against several changes of distilled water at 0°C. The samples thus obtained were freeze-dried in a Virtis Freezedrier. Absorption spectra of the proteins were recorded on a Perkin-Elmer spectrophotometer.

RESULTS

Electrophoresis

Table 1 and Figure 1 show the results obtained on the two fish species. Constant patterns were obtained for both species when using paper electrophoresis (number of fractions) and these patterns are shown in relation to that of man done under similar experimental conditions. In the case of gel electrophoresis a fairly constant pattern was again obtained for both species but variations were, however, evident in the middle third of the electrophoretograms in the percentage protein of various fractions. These are indicated in Figure 1. In all cases the peaks were numbered according to their relative mobilities and not according to the distance moved. These variations could not be correlated with length, weight, sex nor haematocrit and were also not reflected in the paper electrophoretograms.

The mean total plasma protein concentration and haematocrit values in the case of *B. holubi* were 22,2 ± 7,5 mg/ml and 28,9 ± 5,6% respectively and 32,3 ± 10,1 mg/ml and 28,7 ± 4,1% respectively in the case of *C. gariepinus*.

Sodium sulphate precipitations

Precipitation of *B. holubi* plasma with Na_2SO_4 yielded the results shown in Figure 2. Ten per cent Na_2SO_4 caused a removal of fractions 1; 2 and 3 and flattened peaks 5 and 7 in the case of the paper electrophoretogram. Fourteen per cent, 18% and 22% Na_2SO_4 had very little additional effect, apart from smoothing the patterns to some extent. Twenty-six per cent Na_2SO_4

TABLE 1

SUMMARY OF ELECTROPHORETIC RESULTS OF *B. holubi* AND *C. gariepinus* PLASMA PROTEINS

		Distance moved mm		*Relative mobility* %		% *Protein*		*Protein concentration* mg/ml	
		Paper electrophoresis							
		C.g.	*B.h.*	*C.g.*	*B.h.*	*C.g.*	*B.h.*	*C.g.*	*B.h.*
Fractions	1	0,5	0,5	2	3	1,8	2,7	0,63	0,44
	2	3,5	2,5	18	16	3,4	4,5	1,24	0,77
Slowest	3	6,5	5,5	33	34	10,9	7,4	3,53	1,28
	4	9,5	7,0	49	44	20,2	7,7	6,79	1,39
to	5	12,0	8,5	62	53	16,8	16,1	5,28	2,82
	6	13,0	10,0	67	63	11,0	19,5	3,80	3,72
fastest	7	14,0	12,0	72	75	4,7	15,8	1,50	2,97
	8	19,5	14,5	100	90	31,2	16,7	10,22	3,25
	9	—	16,0	—	100	—	9,6	—	1,62
		GEL ELECTROPHORESIS							
Fractions	1	1,5	0,5	3	1	2,1	4,5	0,68	0,95
	2	2,5	1,0	6	2	6,1	1,6	1,87	0,34
	3	3,0	2,5	7	6	3,6	3,5	1,15	0,80
	4	3,5	3,0	8	7	1,3	1,5	0,43	0,31
Slowest	5	4,5	4,0	10	10	1,8	2,4	0,51	0,54
	6	5,0	5,5	12	13	2,3	5,7	0,65	1,32
	7	6,5	7,0	15	17	2,0	3,0	0,58	0,65
to	8	9,0	10,0	21	24	2,9	7,0	0,95	1,60
	9	11,0	11,5	25	27	3,4	7,0	1,04	0,68
fastest	10	12,0	12,0	28	29	3,4	1,9	1,08	0,39
	11	13,5	14,0	31	33	2,0	1,9	0,65	0,40
	12	15,5	15,5	36	37	1,8	7,4	0,59	1,56
	13	17,0	16,5	40	39	2,2	3,5	0,72	0,73
	14	20,0	18,0	46	43	3,0	3,0	0,99	0,66
	15	21,5	21,5	50	51	4,9	5,8	1,57	1,37
	16	23,0	23,5	54	56	4,4	7,5	1,40	1,72
	17	25,5	31,0	59	74	7,0	27,8	2,19	6,23
	18	28,5	38,0	66	90	5,5	6,7	1,73	1,60
	19	30,5	42,0	71	100	7,1	2,0	2,40	0,34
	20	36,5	—	85	—	11,5	—	3,32	—
	21	39,0	—	91	—	19,8	—	5,83	—
	22	43,0	—	100	—	2,4	—	0,75	—

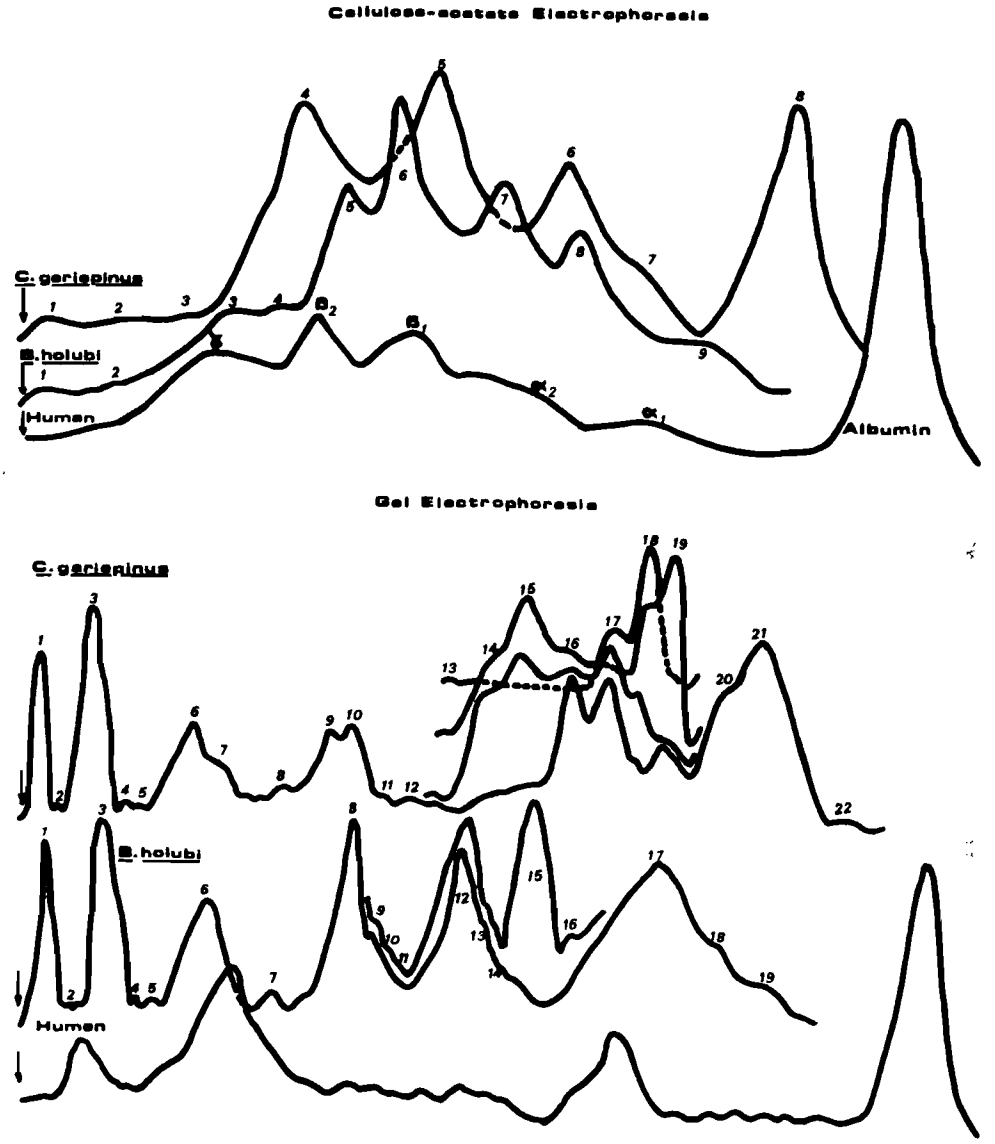


FIGURE 1

Typical electrophoretograms of *B. holubi* and *C. gariepinus* plasma proteins compared to that of man done under the same conditions. Origin on the left (arrows). The variations observed in the case of the gel electrophoretograms are indicated above the standard pattern.

had the most pronounced effect and only peaks 1; 8; 12 and 17 remained (gel electrophoresis), and fractions 6 and 8 in the case of paper electrophoresis.

In view of these results, it was decided to study fractions 8; 12 and 17 more closely.

Separation and analytical results

Four millilitre plasma samples from *B. holubi* were precipitated with 26% Na_2SO_4 and centrifuged. The supernatants were then eluted on Sephadex G-100 columns. The elution pattern obtained is shown in Figure 3A. Above the various peaks, the patterns, obtained from fluid

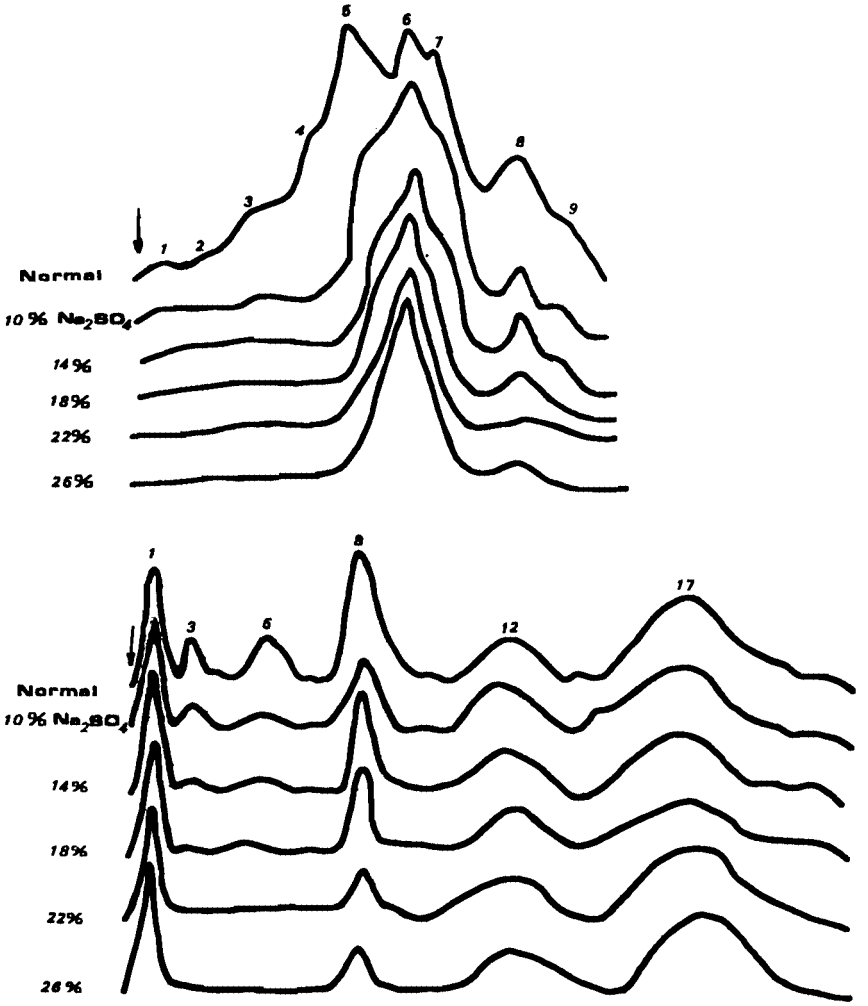


FIGURE 2

Precipitation of *B. holubi* proteins with Na_2SO_4. Supernatant fluid analyzed.

reapplied to polyacrylamide gels, are shown diagrammatically. The tubes containing the desired fractions were then pooled separately (gels 2 and 3 in Figure 3A), dialysed and freeze-dried. At this stage it was found that the sample from gel 3 absorbed strongly at 405 nm and the fraction was discarded.

The remaining sample was then subjected to isoelectric focusing as described. The results obtained are shown in Figure 3B. The two fractions were separated from each other and fraction 12 had an isoelectric point of pH 5,81 and fraction 17 an isoelectric point of about 3,8. The latter pH could not, however, be measured accurately because the fraction was located at the end of the pH gradient. The two fractions were then dialysed, freeze-dried and the absorption spectrum of each was determined. Both showed similar spectra and absorbed strongly at 275 and 220 nm and exhibited minimum absorption at 268 nm.

The column used for initial separation was standardized with alcohol dehydrogenase, cytocrome C, albumin fraction V (bovine), Vit. B_{12} and blue dextran (all from Merck) immediately after the samples had eluted. Approximate average molecular mass of fractions 12 and 17 were then determined. Fraction 12 was found to have an approximate average molecular mass of 55 500 and fraction 17 a value of 89 100. At the same time the two fractions were again placed on 5 % polyacrylamide gels, this time containing 0,1 % SDS. No differences were observed when compared to the distances moved in gels without SDS.

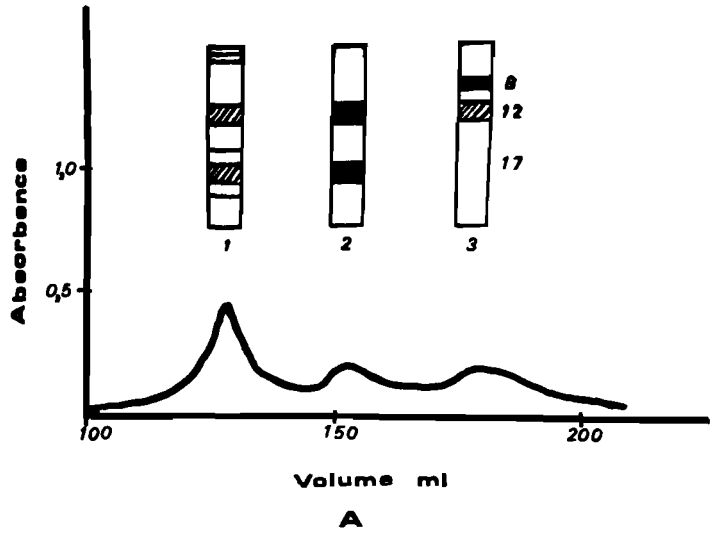
DISCUSSION

The paper electrophoretograms obtained for both species show that each species has a characteristic pattern. This method is, however, not sensitive enough for routine analysis of plasma within one species. For this, gel electrophoresis is far superior (see also Thompson 1967). In this case also, each pattern is again specific enough for each species to be used as a means of identification.

No correlation could be found between the patterns (number of fractions, percentage protein) and sex, gonadal development, body weight or body length (all fish used were mature). Factors such as diet, pollution, age and disease could have influenced the patterns, but these effects were not investigated in this study. The fish used were all from the same locality (within a species) and were all caught during the same season and these two factors could therefore not have influenced the results.

Some of the variations in the polyacrylamide electrophoretograms could have been due to minor experimental differences. These variations, however, were also noted in blood from different animals during the same experiment (eight samples run at the same time) and it therefore appears that they are real; probably a polymorphism (Barret & Tsuyuki 1967).

From the precipitation results on *B. holubi* plasma it is not entirely clear which fraction is precipitated and which is not (albumin or globulin) because it is not possible to equate the electrophoretograms with that of man. Moreover, there are no apparent similarities between yellow-fish plasma protein patterns and those of man (Figure 1). It is therefore not possible to comment on the nature of the albumins and globulins in this species. No precipitation studies were performed on barbel blood and although the paper electrophoretogram bears a resemblance



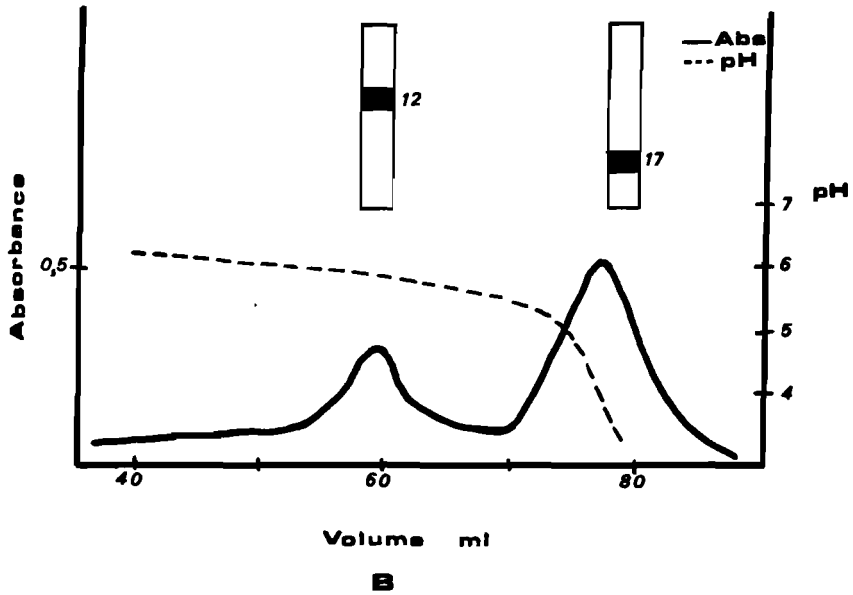


FIGURE 3
A-Elution patterns obtained on Sephadex G-100, Tris-HCl buffer, pH 7,5. B-Isoelectric focusing of sample 2 in A. *B holubi*.

to that of man, the gel pattern is completely different. It is thus again not possible to equate these two. This view is in agreement with that of Mulcahy (1970) and Perrier *et al.* (1973).

The method of approximate average molecular mass determination according to Andrews (1965) is known to have an experimental error of about 10%. It is also known that the sugar content of the proteins influences the results obtained. Anthrone determinations done on samples of the protein available after separation were negative and they are therefore correct to within 10%.

In man, the supernatant fluid obtained after 26% Na_2SO_4 precipitation is known to contain predominantly albumins on electrophoresis (Putnam 1965). According to the human nomenclature therefore, fractions 12 and 17 of *B. holubi* should at least in part be albumins. Neither the molecular masses, nor the isoelectric points of these fractions, correspond to that of human albumin (Putnam 1965) and this further strengthens the concept that the plasma proteins of these fish can only be named after thorough biochemical investigation. The results obtained with the isolated fractions on the SDS-gels at least show that they do not naturally occur in dimeric form (Gordon 1969).

Finally, mention must be made of the fact that in normal, healthy fish of both species the plasma concentrations of haemoglobin are high enough to be shown by the gel electrophoresis technique used (Hattingh 1973). The distance moved in the gels by the haemoglobins corresponds to that of some of the plasma protein fractions. Fraction 8 of *B. holubi* is such a fraction and it probably contains a fair amount of haemoglobin (absorbed strongly at 405 nm). This factor should be borne in mind when studying the plasma proteins with these methods.

ACKNOWLEDGEMENTS

I would like to thank Prof. P. R. Levy and Dr. N. Savage for their advice and criticism.

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