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DOUBLE ELECTROPHORESIS OF THE BLOOD SERUM PROTEINS OF BREAM (*ABRAMIS BRAMA*)

Several investigations have proved that in cyprinid fishes paper electrophoretic protein fractions of the blood serum (or plasma) undergo remarkable quantitative changes connected with some physiological processes (Сорвачев, 1957; Barnoud, Pérès, 1964; Кирсипуу, 1964; Kirsipuu, 1971b; Иванова, 1973). However, the interpretation of the changes observed turned out to be very difficult (except for albumins and α_2-globulins) as the actual composition and functions of paper electrophoretic fractions in fishes are factually unknown so far. The scheme of human plasma proteins is presumed to be applicable in general (Kulow, 1966) but concrete data are lacking. Therefore, some misleading conclusions can be made. For example, by analogy with man, the quantitative changes in β-globulin fraction are supposed to be connected with the changes in lipid metabolism (Иванова, 1973), although it is known that in cyprinid fishes the β-globulin fraction of blood serum proteins does not contain any lipid component (Kirsipuu, 1971a).

As we have shown in an earlier paper (Kirsipuu, 1975), in the common carp blood serum protein electrophoregram in polyacrylamide (PAA) gel is, in principal, similar to that in starch gel as described by R. Creyssel and collaborators (1964). On the other hand, the disposition of several fractions is, to some extent, comparable to those in human plasma proteinogram (Dugne et al., 1972). The aim of the present paper is to check the applicability of the scheme of the carp serum protein pattern to the blood serum protein system of an another cyprinid fish, *Abramis brama*.

Material and methods

Seven bream specimens from Lake Võrtsjärv (Estonian SSR) were investigated: at the end of April 1974 (3 sp.), in November 1974 (2 sp.) and in March 1977 (2 sp.). Two of them were immature females, four mature females and one mature male. The fish were caught by net and analyzed immediately after their conveying to the laboratory, while in November 1974 they were kept in a reservoir for twenty-four hours at the water temperature of 6 °C.

The technique of obtaining serum and carrying out electrophoresis both at filter paper strips and in PAA gel was the same as described previously (Kirsipuu, 1975), except for the run time by paper electrophoresis, which was 12 hours in this case.

Some bands at the PAA gel electrophoregrams were rather weak,

therefore it was not expedient to present the photographs. The same is mentioned by J. McKenzie and U. Paim (1969) as well as by A. Gruzdev and collaborators (Груздев et al., 1972).

In the schemes presented here the relative intensity of the bands is roughly reflected in the density of shading.

Results

By means of paper electrophoresis the blood serum proteins of the bream can be separated into five fractions, conventionally designed as albumin, α_1-, α_2-, β- and γ-globulins. Sometimes the separation of β-globulins into two fractions is observed (Кирсипуу, 1964). By means of PAA gel electrophoresis up to 20 fractions (hencefore called «bands» when discussing PAA gel electrophoretic data, to avoid misunderstanding) can be detected in this fish (Яаска, Кирсипуу, 1971) as also proved by the results of the present experiment.

After double electrophoresis in which the paper electrophoretic fractions were separately analyzed by means of the PAA gel electrophoresis, several bands were separated from each fraction (Fig. 1), except for the albumin which was the only really homogeneous paper electrophoretic fraction.

The paper electrophoretic α_1-globulin fraction appeared to consist of two prealbumin bands and of a high molecular weight protein. In some cases, some weak bands were observed in the postalbumin zone. However, it was not always clear if they were really different from the traces of neighbouring α_2-globulins as in PAA gel tubes the moving speed can be slightly different and negligible differences in the disposition cannot always be taken into account.

The further separation of the α_2-fraction revealed an important band running hardly slower than the albumin. At the PAA gel proteinograms of the whole serum this band indiscernibly blended with albumin in most cases. Besides that, one or two consistent bands were always detected in the zone of postalbumins and, in some cases, rather weak and slow-moving bands were detected. In females with ripening roe (the IV stage of development of gonads) a very strong slow band was always observed.

As in the carp, the β-globulins in the bream were also the most heterogeneous fractions. Usually two bands of intermediate moving speed and some bands of low speed could be detected.

In one case we succeeded in analyzing the β_1- and β_2-globulins separately. This experiment revealed that the fastest of the bands of intermediate moving speed was the β_1-globulin, the others being β_2-globulin. Slowly moving bands were β_1-globulins while the slowest component seemed to be β_2-globulin, a noticeable trace of which was detectable in the β_1- and γ-fraction, too.

The γ-globulin fraction revealed, after repeated electrophoresis in PAA gel, only one specific band and some evident traces from the β-fractions in most cases. However, another specific band was detected in the specimen N 10, Apr. 29th, 1974 (Fig. 1d). As this band was absent at the PAA gel proteinogram of the whole serum in this specimen (see Fig. cited above), we cannot declare it being a characteristic γ-globulin component.

The general scheme of the intercorrespondence of paper electrophoretic and gel electrophoretic blood serum protein fractions in the bream can be drawn as presented in Fig. 2.

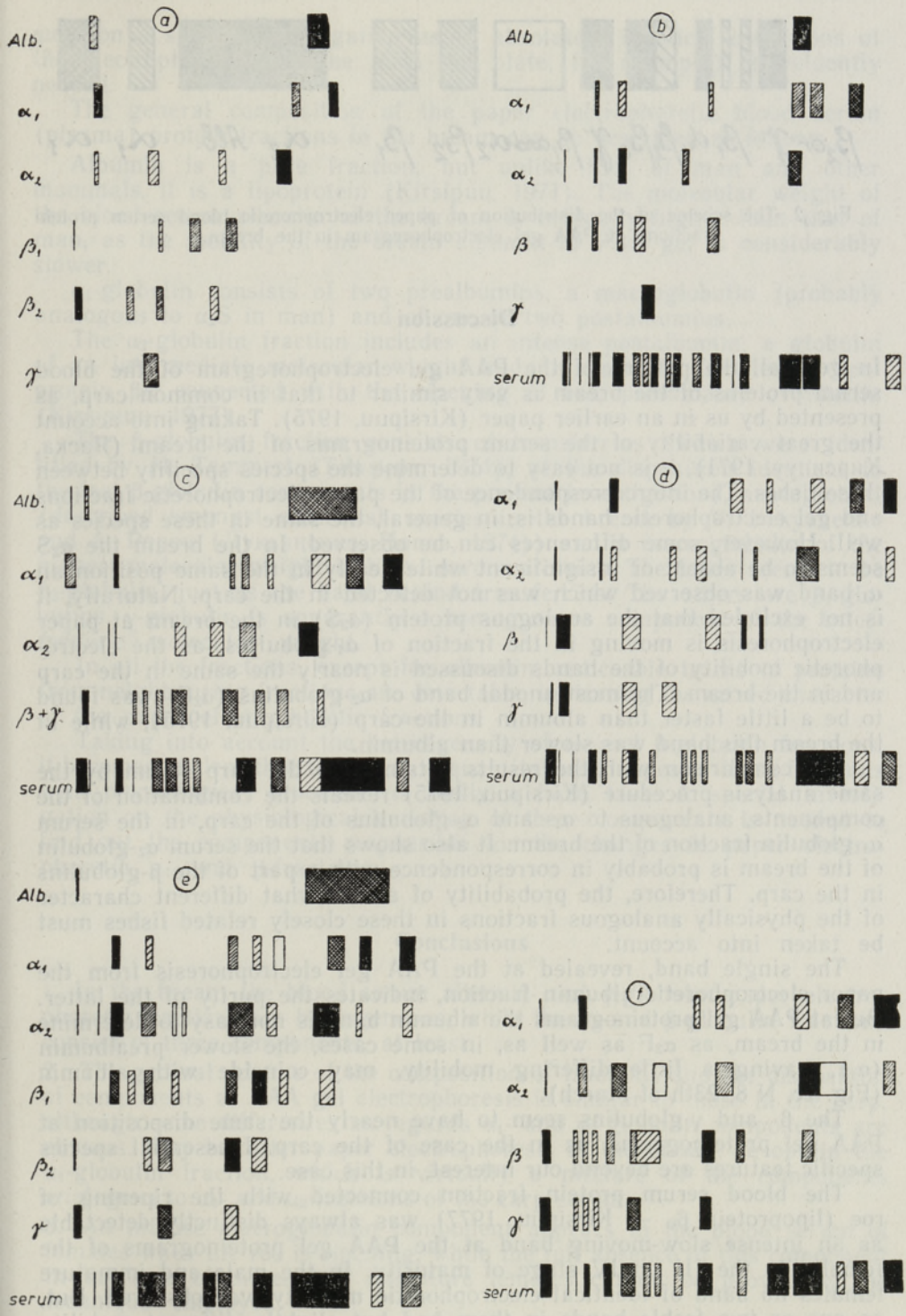

Fig. 1. The schemes of PAA gel electrophoregrams of the paper electrophoretic blood serum protein fractions in some specimens of the bream; a) ♀ II N 1, Nov. 30, 1974; b) ♀ III N 3, Nov. 30, 1974; c) ♀ II N 8, Apr. 29, 1974; d) ♂ IV N 10, Apr. 29, 1974; e) ♀ IV N 8, March 23, 1977; f) ♀ IV N 10, March 23, 1977.

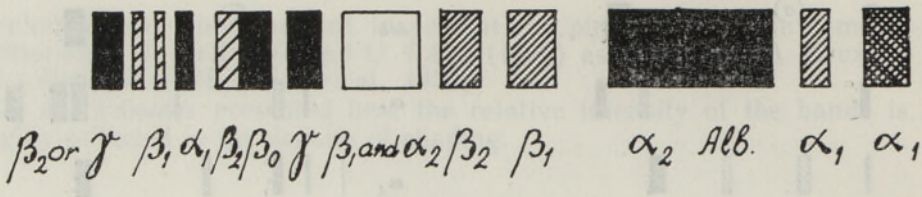

Fig. 2. The scheme of the distribution of paper electrophoretic blood serum protein fractions at PAA gel electrophoregram in the bream.

Discussion

In general, the pattern of the PAA gel electrophoregram of the blood serum proteins of the bream is very similar to that in common carp, as presented by us in an earlier paper (Kirsipuu, 1975). Taking into account the great variability of the serum proteinograms of the bream (Яаска, Кирсипуу, 1971), it is not easy to determine the species specifity between these fishes. The intercorrespondence of the paper electrophoretic fractions and gel electrophoretic bands is, in general, the same in these species as well. However, some differences can be observed. In the bream the α_2S seems to be absent or insignificant while nearly in the same position an α_1-band was observed which was not detected in the carp. Naturally, it is not excluded that the analogous protein (α_2S) in the bream at paper electrophoresis is moving in the fraction of α_1-globulins, as the electrophoretic mobility of the bands discussed is nearly the same in the carp and in the bream. The most anodal band of α_2-globulins (α_2F) was found to be a little faster than albumin in the carp (Kirsipuu, 1975), while in the bream this band was slower than albumin.

The comparison with the results obtained on the carp serum by the same analysis procedure (Kirsipuu, 1975) reveals the combination of the components, analogous to α_1- and α_2-globulins of the carp, in the serum α_1-globulin fraction of the bream. It also shows that the serum α_2-globulin of the bream is probably in correspondence with a part of the β-globulins in the carp. Therefore, the probability of a somewhat different character of the physically analogous fractions in these closely related fishes must be taken into account.

The single band, revealed at the PAA gel electrophoresis from the paper electrophoretic albumin fraction, indicates the purity of the latter. But at PAA gel proteinograms the albumin band is not easy to determine in the bream, as α_2F as well as, in some cases, the slower prealbumin (α_1), having a little differing mobility, may coincide with albumin (Fig. 1c, N 8, 23th of March).

The β- and γ-globulins seem to have nearly the same disposition at PAA gel proteinograms, as in the case of the carp. Inessential species specific features are beyond our interest, in this case.

The blood serum protein fraction connected with the ripening of roe (lipoprotein β_o — Kirsipuu, 1977) was always distinctly detectable as an intense slow-moving band at the PAA gel proteinograms of the females in the III an IV stage of maturity. In the male and immature females no band of identical electrophoretic mobility was observed. But, as one or two feeble bands in them had but slightly different mobility from β_o, and, keeping in mind the possibility of a difference of moving speed in tubes, a complete absence of the analogous protein in male and immature female cannot be confirmed categorically. To answer this

question a special investigation using absolutely identical conditions of the electrophoresis (at the PAA gel plate, for example) is evidently needed.

The general composition of the paper electrophoretic blood serum (plasma) protein fractions in the bream can be described as follows.

Albumin is a pure fraction, but unlike that of man and other mammals, it is a lipoprotein (Kirsipuu, 1971). The molecular weight of the blood serum albumin of the bream is evidently higher than that of man, as the mobility of the bream albumin in PAA gel is considerably slower.

α_1-globulin consists of two prealbumins, a macroglobulin (probably analogous to α_2S in man) and of one or two postalbumins.

The α_2-globulin fraction includes an intense postalbumin, a globulin of an intermediate molecular weight and, in maturing females, a lipoprotein β_o, connected with the ripening of roe, presumably ovovitellin (Kirsipuu, 1977).

The β-globulin fraction consists of transferrins (which were identified by M. Tammert (Таммерт, 1974), at the disposition identical to the fast β-globulin components in the present investigation), haptoglobins (described amongst other fish species in the bream by V. Lukyanenko and A. Popov (Лукьяненко, Попов, 1974)) and a macroglobulin of the highest molecular weight in blood serum proteins. The latter seems to be β_2-globulin, just as the band behind transferrins. The others are β_1-globulins. γ-globulin is apparantly a homogeneous component of the intermediate molecular weight.

In all the fractions, except for albumin, noticeable traces of proteins from neighbouring fractions are detectable, indicating the mixed character of the paper electrophoretic fractions.

Taking into account the heterogeneity of α- and β-globulin fractions, it becomes evident that to associate quantitative changes in these fractions with physiological processes is really a hard task. A special investigation on the physiological variation of these components is needed to find out the components responsible for the changes in paper electrophoretic α- and β-fractions.

Conclusions

1. In the bream the blood serum albumin and γ-globulin paper electrophoretic fractions are homogeneous, while the α- and β-globulin fractions consist of three components, at least.

2. The general scheme of the composition of fractions and the disposition of components at PAA gel electrophoresis is similar to those in the carp, although some differences as regards α_1- and α_2-globulin components are obvious. May be, after paper electrophoresis in the bream we obtain the α_1-globulin fraction, which is virtually a mixture of the components analogous to α_1- and α_2-fractions of the carp serum.

3. An intense macroglobulin component appears in the blood serum of females when the roe is ripening, while the full absence of this component in the sera of males and immature females cannot be proved by the technique employed.

4. The paper electrophoretic α- and β-globulin fractions are obviously polyfunctional. Their role in physiological processes requires a special investigation. Attempts to explain changes in these fractions by a single physiological process will evidently be unjustified.

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LATIKA (*ABRAMIS BRAMA*) VERESEERUMI VALKUDE KAHEKORDNE ELEKTROFOREES

Resümee

Latika vereseerumist paberelektroforeesi teel saadud valgufraktsioonide edasisel elektroforeesimisel polüakrüülamiidgeelis osutusid homogeenseteks albumiin ja γ-globuliin. α- ja β-globuliinide fraktsioonid koosnesid vähemalt kolmest komponendist; nendes leidus ka tähelepandaval hulgal naaberfraktsioonide jälgi.

Paberelektroforeetiliste valgufraktsioonide koostis ja nende komponentide liikuvus elektroforeesimisel polüakrüülamiidgeelis oli latikal üldjoontes sarnane karpkala vastavate näitajatega. Olulisemad erinevused ilmnesid α_1- ja α_2-globuliinide fraktsioonides, milles osa komponente oli karpkalaga võrreldes vahetunud.

Emaste latikate vereseerumi α_2-globuliinide fraktsiooni ilmus marja valmides rohkesti spetsiifilist makroglobuliinset komponenti. Selle täielikku puudumist isaste ja mittesuguküpsete kalade veres aga ei võimaldanud kasutatud meetod tõestada.

Käesoleva uurimuse tulemusena ilmnenud latika vereseerumi α- ja β-globuliinide heterogeensus viitab nende polüfunktsionaalsusele. Sellest järeldub, et katsed selgitada nende kvantitatiivseid muutusi mingi ühe füsioloogilise protsessi toimel on vähe põhjendatud.

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ДВОЙНОЙ ЭЛЕКТРОФОРЕЗ БЕЛКОВ СЫВОРОТКИ КРОВИ ЛЕЩА (*ABRAMIS BRAMA*)

Резюме

При повторном электрофорезе в полиакриламидном геле бумажно-электрофоретических фракций белков сыворотки крови леща гомогенными оказались альбумин и γ-глобулин. Фракции α- и β-глобулинов содержали не менее чем три компонента. В этих фракциях в значительной степени обнаруживались и следы соседних фракций.

У леща состав и подвижность бумажно-электрофоретических фракций были в общих чертах похожими на состав и подвижность фракций у карпа. Более значительные различия обнаружены во фракциях α_1- и α_2-глобулинов, где у леща некоторые компоненты распределялись несколько иначе, чем у карпа.

В α_2-глобулиновой фракции сыворотки крови самок леща во время созревания икры появлялся в значительном количестве специфический макроглобулиновый компонент. Однако доказать полное отсутствие его у самцов и неполовозрелых рыб с помощью использованного метода также не удалось.

Выясненная в результате настоящего исследования гетерогенность α- и β-глобулинов сыворотки крови леща указывает на их полифункциональность. Следовательно, попытки объяснить количественные изменения в названных функциях на основе отдельного физиологического процесса являются мало обоснованными.

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