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ON THE ELECTROPHORETIC PROTEIN FRACTION, CONNECTED WITH THE MATURING OF ROE, IN THE BLOOD SERUM OF FISHES

As a result of the investigations of the electrophoregrams of blood serum (or plasma) proteins of fishes, several authors have drawn attention to differences between sexes. The first to mention it was A. Drilhon (1954). Later most of the authors have confirmed the existence of differences between sexes. Only in very few cases their absence has been mentioned (Nyman, 1965a, b, 1967; Mulcahy, 1970; Исаев et al., 1969; Недина, 1971a). In the course of seasonal studies it has been determined that the differences arise so to speak unilaterally in the blood of females in connection with the maturing of roe (Einszporn-Orecka, 1970; Головки, 1964; Кирсипуу, 1964a, b; Кирсипуу, Пиху, 1965; Шатуновский, 1967). Therefore we have good reason to assume that the above-mentioned difference in opinions has been caused by the fact that some authors happened to study the periods when gonads of females were in the state of rest (Исаев et al., 1969; Недина, 1971a) or the roe was at the initial stage of development, and differences between sexes had thus not been yet formed.

In studies, the aim of which has been the comparison of species or intraspecific groupings, researchers have often confined themselves to the stating of differences between sexes (Drilhon, 1954; Yamashita, 1968a, 1969; Остроумова, 1969) without any specification of the differences. A comparison of several authors' studies reveals that in the case of the electrophoresis with poor resolving power, the difference mainly appears in the increase of the importance of one globulin fraction (Yamashita, 1968b; Pesch, 1970; Moreau, 1972; Галасун, Шемчук, 1971). Some authors have found the amount of α -globulins to increase (Кузьмина, 1968; Шатуновский, 1967; Шатуновский et al., 1967; Квасова, 1968). When it was possible to divide the fraction into subfractions, an increase was observed in α_2 -globulins (Lecal, 1958; Einszporn-Orecka, 1970; Kirsipuu, 1971b; Кирсипуу, 1964a, b; Кирсипуу, Пиху, 1965; Литвинова, 1968; Барсук, Зименко, 1971). Other investigators have found that in connection with the maturing of roe the fraction of β -globulins of females increases (Yamashita, 1968b; Головки, 1964; Остроумова, 1967; Литвинова, 1968; Недина, 1971b; Ирискин, 1972; Муромцева, 1974).

Different standpoints of investigators can be explained by two kinds of reasons.

On the one hand, it is quite obvious that the division of protein fractions of the blood serum of fishes into α - and β -globulins is rather arbitrary. As great differences occur in the mobility of these fractions in different fishes and as the mobility of them in several fishes is noticeably different from that of the protein fractions of man's blood serum

(Mulcahy, 1970; Кирсипуу, 1964a, 1974), it is impossible to identify separate fractions and subfractions by means of comparison of phoregrams. It is rather difficult to place subfractions into groups of globulins and even to limit the fraction (or fractions) of albumins. An application of chemical tests has not been of any great use either (Snieszko et al., 1966). Therefore different authors have obviously sometimes marked the same fraction differently (cf. Кирсипуу, 1964a; Литвинова, 1968; Недина, 1971a, b).

On the other hand, it is clear that in the case of fishes belonging to different families and orders, the globulin connected with the maturing of roe has a different electrophoretic mobility and a different relative location on the proteinogram. For example, this protein of the pike-perch moves approximately as quickly as man's α_2 -globulin. However, at least four fractions are quicker than this one (Кирсипуу, 1964a). As for the pike, its fraction with the same function lies in the position of β -globulins although it occupies only the third place as to the speed of motion (Fig. 1).

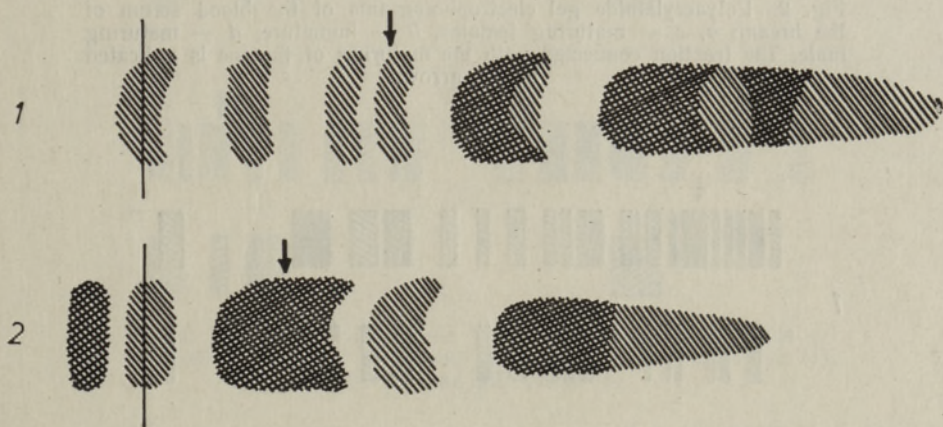


Fig. 1. Localization of the maturing-connected fraction (indicated by the arrow) at the paper-electrophoretic blood serum proteinogram of the pike-perch (1) and pike (2).

As our studies have mostly treated fishes belonging to the family of cyprinids, in whose case the fraction under observation lies in the α_2 -position on the paper electrophoregram, we have also considered the analogical fraction of other fishes in our investigations as α_2 -globulin (Кирсипуу, 1964a, b, 1966; Кирсипуу, Пиху, 1965).

Electrophoresis in gels with a better resolving power (starch, polyacrylamide) still makes some specifications possible. Investigations of several authors enable us to conclude that gel electrophoregrams are, in principle, similar to those of man, and several fractions can easily be identified (prealbumins, albumins, transferrins, α_2S -globulin, etc. — Drilhon, 1960; Creyssel et al., 1964; Salibian, 1965; Лукьяненко et al., 1967; Груздев et al., 1972). On such proteinograms some authors have found that females with maturing roe have an additional fraction (in some cases several fractions) in the region of α_2 -macroglobulin (α_2S) (Thurston, 1967; Komatsu et al., 1970; Uva, 1971) while others stress big changes in connection with the maturing of gonads (Thurston, 1967; Седов, Лукьяненко, 1969). Our study, carried out on the basis of a comparatively scanty material on the bream, did not reveal any distinct differences between females and

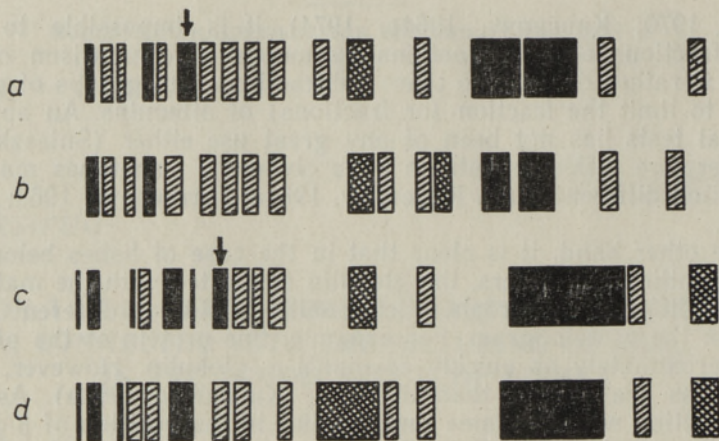


Fig. 2. Polyacrylamide gel electrophoregrams of the blood serum of the bream: *a*, *c* — maturing females, *b* — immature, *d* — maturing male. The fraction connected with the maturing of the roe is indicated by the arrow.

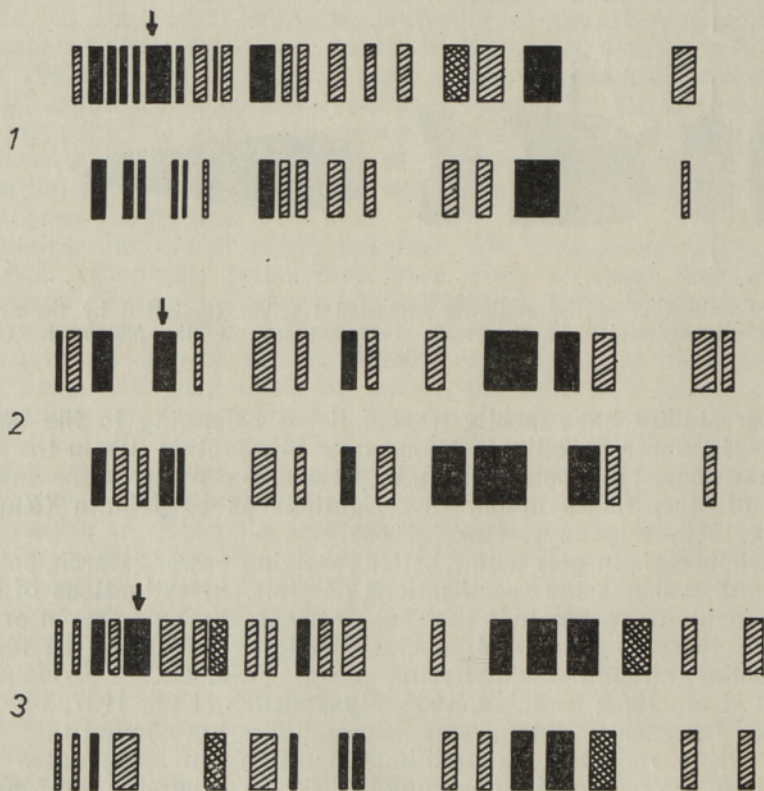


Fig. 3. Polyacrylamide gel proteinograms of the pike (*1* — female and male), burbot (*2* — female and male) and pike-perch (*3* — female and immature).

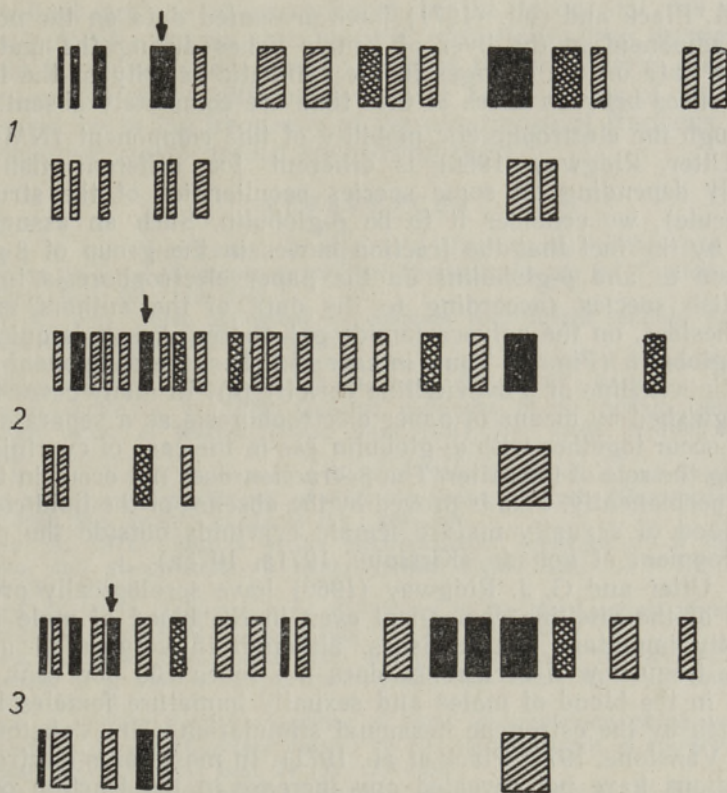


Fig. 4. Protein and lipoprotein fractions at the polyacrylamide gel electrophoregrams of the blood serum of the female burbot (1), pike (2) and pike-perch (3).

males in the polyacrylamide gel proteinograms of the fish (Яаска, Кирсипуу, 1971). Later additional studies of bulkier material still proved that during the transition of gonads from stage II (VI) of sexual maturity to stage III the amount of protein in the fraction located towards the anode from α_2 -S-globulin increases in females. Therefore this fraction becomes especially strong (Fig. 2). The appearance of a similar strong fraction was also observed in the females of some other fish species (pike-perch, pike, burbot) although its relative position on the proteinogram was somewhat different from that of the bream (Fig. 3). The colouring of phoregrams by Sudan Black B revealed that the fraction is sudanophile (Fig. 4). On paper electrophoregrams the fraction of females connected with the maturing of roe was also found to contain lipoproteids (Кирсипуу, 1971а; Кирсипуу, 1975а; Ван Цзу-сюн, Ван Цзинь-бао, 1964; Куликова, 1967). This allows us to conclude that it is functionally one and the same protein. J. R. Markert and W. E. Vanstone (1971) have separated it both from the blood and roe of the coho salmon and found it to be analogical to the lipovitelline of birds. An identical lipoprotein in the blood and roe of female fishes has also been found by some other investigators (Utter, Ridgway, 1966; Plack et al., 1971). The above-said justifies an assumption that during the maturing of roe a special lipoprotein appears in the blood of all female fishes, by means of which the ovovitelline synthesized in the liver is transported to the roe (Utter, Ridgway, 1966). According to the information at our disposal,

only P. A. Plack and coll. (1971) have presented data on the occurrence of this component in the liver of female fishes during the maturing of roe, while data on the changes in the synthetic activity of the liver and the differences between sexes at that time are completely absent.

Although the electrophoretic mobility of this component (NM-component — Utter, Ridgway, 1966) is different for different fish species (obviously depending on some species peculiarities of the structure of the molecule), we consider it to be β -globulin. Such an assumption is justified by the fact that the fraction moves in the group of β -globulins or between α - and β -globulins on the paper electrophoresis in case of several fish species (according to the data of the authors mentioned above); besides, on the polyacrylamide gel phoregrams it is quicker than α_2 -macroglobulin (Fig. 2). Thus, in case of fishes, the component could be called β -lipovitelline or β -ovovitelline (briefly β_0). In many cases it cannot be distinguished by means of paper electrophoresis as a separate fraction and may occur together with α_2 -globulin (as in the case of cyprinids), thus increasing the role of the latter. The β_0 -fraction does not occur in the blood of fishes permanently. This is proved by the absence of the lipid component in the blood of sexually mature female cyprinids outside the period of the development of gonads (Kirsipuu, 1971a, 1975a).

F. M. Utter and G. J. Ridgway (1966) have serologically proved the existence of the vitelline component even in the blood of male and also in sexually immature female fishes, although in very small quantities while the frequency of occurrence does not reach 100 per cent. Its appearance in the blood of males and sexually immature females has been called forth by the estrogene hormonal stimulation (Ho, Vanstone, 1961; Markert, Vanstone, 1971; Plack et al., 1971). In most cases electrophoretic investigations have not revealed any increase in the fraction connected with the maturing of roe in the blood of males in connection with the maturing of the gonad. Some authors have still found that the corresponding protein fraction changes even in the blood of male fishes in connection with the sexual cycle (Einszporn-Orecka, 1970; Литвинова, 1968).

Our earlier studies on the bream revealed only an inessential rise of the percentage of α_2 -globulins in males and sexually immature fishes during the prespawning period (Kirsipuu, 1971b). We considered it an accidental phenomenon. However, subsequent analysis of over 100 male breams during the spawning period revealed a high number of specimens in the blood of which the percentual content of α_2 -globulins had considerably increased. Therefore it is highly possible that during a short period of time a protein identical or similar to ovovitelline, essential for the final maturing of milt, is formed in the blood of male fish. However, according to our data, this fraction of males never contains a lipid component. Neither can we, on the basis of proteinograms obtained in polyacrylamide gel, assert categorically that the protein component of the β_0 -fraction is fully absent in the blood of males and immature fish as several fractions of very similar mobility can be distinguished in this area. Their comparison on the proteinograms of different fish is rather difficult. And, besides, we have to take into account a possibility that the electrophoretic mobility of the (whole) fraction may change due to the addition of the lipid component. A. Salibian (1965) has also referred to the possibility.

In addition, the analysis of the electrophoregrams of the polyacrylamide gel showed that differences between sexes probably exist in some other weaker globulin fractions as well (Fig. 3). It was especially noticeable in the case of the pike-perch. It is no surprise as besides lipovitelline (ovovitelline) J. R. Markert and W. E. Vanstone (1971) have found one

more identical protein (β' -component) in the blood and roe of fishes, and it is generally known that specific globulin-binding estrogenic hormones can be found in the blood serum of fishes (Martin, Ozon, 1971). However, as the above differences do not seem to be of an absolute character but lie in the frequency of the occurrence of fractions, statistically reliable material is needed for proving them.

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MARJA VALMIMISEGA KAASNEVAST ELEKTROFOREETILISEST VALGUFRAKTSIOONIST KALADE VERESEERUMIS

Resümee

Kalade vereseerumist mitmesuguste elektroforeesimeetodite abil saadud proteinoogrammide võrdlemisel ilmneb, et suguküpsete emaste verre ilmub marja valmimise ajal eriline lipiidset komponenti sisaldav fraktsioon. See on ilmselt lipovitelliin (ovovitelliin), mida on leitud ka marjaterade rebus. Tema elektroforeetiline liikuvus on eri kalaliikidel erinev. Mõnel liigil paikneb ta α_2 -globuliinide, teistel β -globuliinide piirkonnas. Ta on tuntav funktsionaalse muutlikkuse tõttu ning tema tähiseks võiks kõigi kalaliikide puhul olla β_0 (β -ovovitelliin). Võimalik, et selle fraktsiooni valgulist komponenti leidub ka isaste ja peatselt suguküpseks saavate emaste kalade veres.

Peale selle fraktsiooni näib veel mõningaid nõrku fraktsioone olevat valmiva marjaga emaste kalade vereseerumis sagedamini kui isaste ja mittesuguküpsete kalade vereseerumis.

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О СВЯЗАННОЙ С СОЗРЕВАНИЕМ ИКРЫ ЭЛЕКТРОФОРЕТИЧЕСКОЙ БЕЛКОВОЙ ФРАКЦИИ В СЫВОРОТКЕ КРОВИ РЫБ

Резюме

При сравнении протеинограмм сыворотки крови рыб, полученных различными авторами с использованием разных методов электрофореза, обнаружено, что в крови у половозрелых самок во время созревания икры появляется специфическая фракция, содержащая липидный компонент. Это, явно, липовителлин (ововителлин), найденный и в желтке икринок. Электрофоретическая подвижность этой фракции у разных видов рыб различна. У некоторых видов она локализуется в зоне α_2 -глобулинов, а у некоторых — в зоне β -глобулинов. Эту фракцию можно узнать по функциональной изменчивости, и у всех видов рыб ее следовало бы обозначить как β -ововителлин (β_0). Возможно, белковый компонент этой фракции содержится и в крови самцов и недавно созревших самок.

Кроме этой фракции, некоторые слабые фракции содержатся в сыворотке крови самок с созревшей икрой, видимо, чаще, чем в сыворотке крови самцов и неполовозрелых особей.

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