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DOUBLE ELECTROPHORESIS OF THE BLOOD SERUM PROTEINS OF PIKE-PERCH (*LUCIOPERCA LUCIOPERCA*)

By means of paper electrophoresis the blood serum proteins of the pike-perch can be separated into up to 8 fractions (Кирсипуу, 1964a). However, in a great number of experiments we could not achieve such a perfect separability, although attempts were made to avoid differences in the conditions of analyses. Some investigators using the same methods have obtained different results (Кузьмина, 1968 — 5 fractions; Литвинова, 1968a, b — 5...7 fractions). Comparing the percentages of the fractions presented by the authors, we can see that they do not coincide even in general lines (Кирсипуу, 1964a, 1966; Литвинова, 1968a,b). It is evident, therefore, that several quantitatively inessential components are blended to different main fractions, possibly depending on the slight differences in the methods or on the physiological state of the fish investigated. This very likely happens due to the really great number of different protein components in the serum of the pike-perch, reaching 33, as indicated by V. Lukyanenko and A. Popov (Лукьяненко, Попов, 1971). Moreover, the conventional designation of the fractions does not evidently coincide in our papers and those by N. Litvinova, cited before. As albumins we have discussed the two most anodal fractions, while N. Litvinova has, apparently, taken only the most distal ones for the albumin. Therefore the percentage of albumins is considerably different, and the comparison of the percentages of other fractions is difficult.

As compared with other fishes, the paper electrophoregram of the blood serum proteins of the pike-perch is noticeably different. It is, to some extent, only comparable with that of the rainbow trout where fast-moving fractions are also prevailing and two (or even three) albumin-like fractions have been detected (Кирсипуу, 1974).

Therefore additional data on the composition of the paper electrophoretic blood serum protein fractions of the pike-perch seems to be necessary.

Material and methods

The blood sera of three sexually mature pike-perches, caught by net in Lake Võrtsjärv (Estonian SSR) at the end of January, 1977, were investigated. N 1 was female, N 2 and 3 were males. The serum proteins were separated into fractions by means of paper electrophoresis. Subsequently each fraction was separately subjected to electrophoresis in polyacrylamide (PAA) gel, as described in a former paper (Kirsipuu, 1975).

To avoid misunderstandings, the paper electrophoretic fractions will be discussed as «fractions» and those of PAA gel proteinograms as «bands».

As the majority of faint bands at PAA gel proteinograms did not appear distinctly enough in photographs, only schemes are presented here.

Results

By means of paper electrophoresis 8 protein fractions, in PAA gel up to 22 bands, were distinctly separated from the pike-perch blood serum (Figs 1, 2). Individual variation in the PAA gel proteinograms was remarkable (Fig. 2). In general, the pattern was comparable to the one presented by V. Lukyanenko and A. Popov (Лукьяненко, Попов, 1971) although some differences were evident in the central and slow zones.

The separation of paper electrophoretic fractions in PAA gel led to the results presented in Fig. 2.

The most anodal fraction (A_1, albumin-1) was found to consist of a quickly moving component and a very slow one. At the PAA gel proteinogram of the whole serum the latter was the second from cathode.

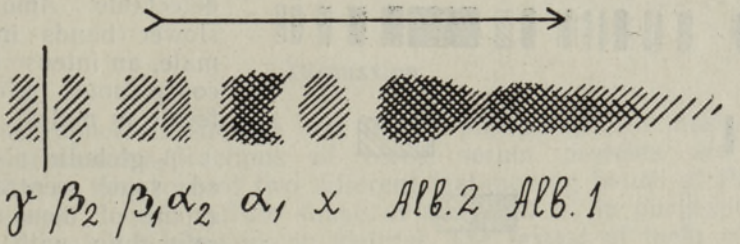


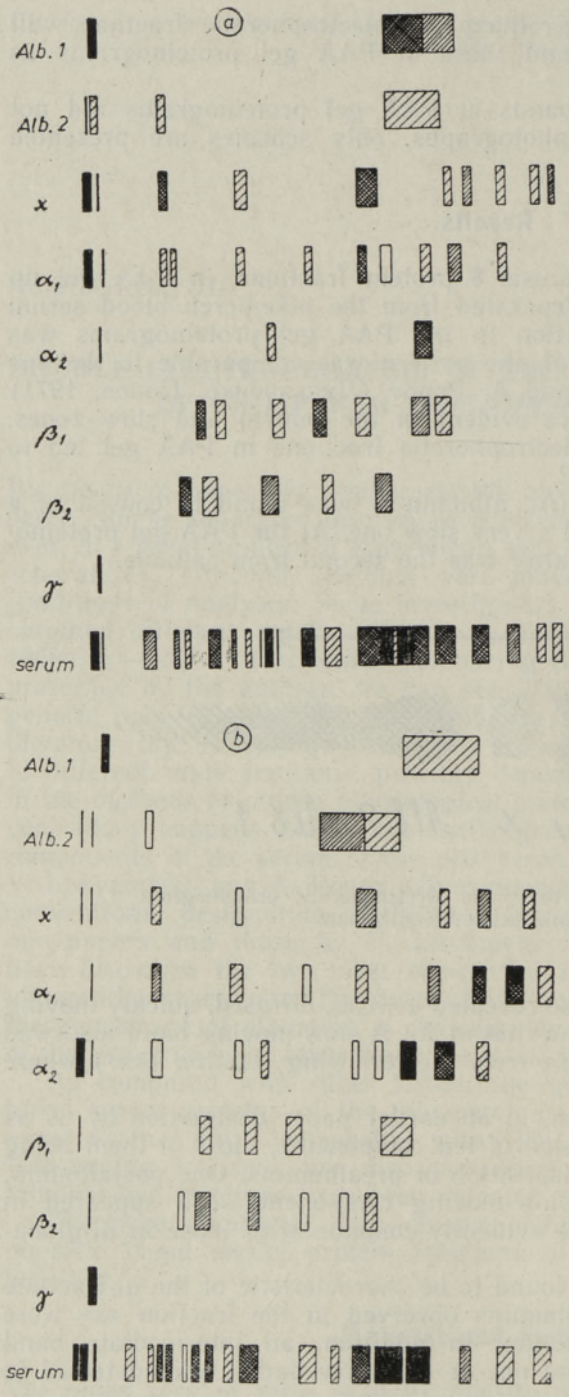
Fig. 1. The scheme of the paper electrophoretic proteinogram of the pike-perch blood serum.

Fraction A_2 (albumin-2) also revealed a weak, diffused, quickly moving band. It was a little slower than that of A_1. A slow-moving band also was detected, but it could have come from the following fraction («x») where such a band was distincter.

The following weak fraction, in an earlier paper designated by us as «x» (Кирсипуу, 1964a), consisted of ten components, most of them being fast moving and having the disposition of prealbumins. One postalbumin, one middle band and three slow-moving components also appeared in this fraction. Some bands were evidently common with those in α_1-globulin.

One prealbumin band was found to be characteristic of the α_1-fraction. Distinct traces of other prealbumins observed in the fraction «x» were also detectable in the α_1-fraction. In addition, an intermediate band coinciding with that in «x» and one or two slow bands were detected in this fraction.

In males the α_2-globulin paper electrophoretic fraction revealed two bands at PAA gel electrophoresis moving as quickly as the faster bands of A_1 and A_2. However, they were distincter and intenser than the latter. In the female only one band of them, the slower, was discovered in this zone. An intermediate band was also detectable both in males and in the female. In addition there was a very slow component in males.



β_1-globulin was very heterogeneous, revealing 7...8 components, most of them being in the middle part of the proteinogram. Surprisingly some of them appeared to be identical to some components in «x» and α_1-fractions, as regards their electrophoretic mobility in PAA gel. In the α_2-fraction these components were not discovered. In the female two distinct bands moving as fast as albumins were likewise observed in β_1-globulin, while in analogous disposition in males a very weak shadow only was detectable. Amongst the slower bands in the female, an intense band was conspicuous, which was feeble in males.

β_2-globulin also revealed some central bands (some of them obviously coinciding with those in β_1) and, once again, some of them (not discovered in β_1) coincided with bands from faster fractions. In this fraction, too, in the female an intense slow band was evident, differing from that in β_1. Surprisingly, no bands of comparable intensity were detectable at the PAA gel proteinogram of the whole serum of the female in the disposition of «female-specific» slow β-bands (Fig. 2).

The paper electrophoretic γ-globulin fraction was homogeneous indeed: the only band was the slowest one at PAA gel electrophoresis, as well.

Obviously, several faint bands were not discovered in paper electrophoretic fractions because of dilution. In addition, some bands appearing in paper electrophoretic fractions were absent at the PAA gel proteinograms of the whole serum. Among them, the most noticeable and

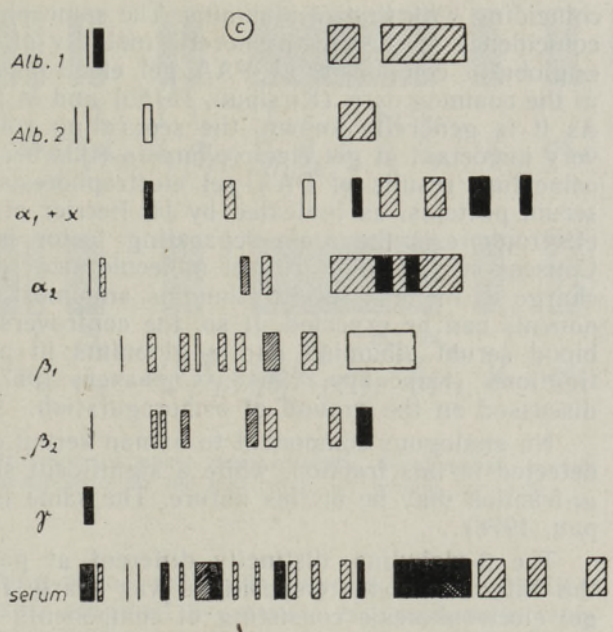


Fig. 2 Schemes of the PAA gel electrophoregrams of the paper electrophoretic blood serum protein fractions in pike-perches; a) ♀ III, N 1; b) ♂ III, N 2; c) ♂ III, N 3.

slowest band from «x» or α_1 was suspectable at the whole plasma proteinogram. Some bands seemed to have changed their electrophoretic mobility in PAA gel if they were gained from the extract of a single paper electrophoretic fraction (β_2 in N 3, Fig. 2, for example).

Discussion

Double electrophoresis showed that in the pike-perch two most anodal paper electrophoretic fractions of blood serum proteins are really albumin-like as they formed two different fast-moving bands at PAA gel electrophoresis. Such a diffuse shape of the band is, in our experience, characteristic of fish albumins in general. The fastest of them at paper electrophoresis was likewise the fastest of the two at PAA gel electrophoresis.

Thus, taking into account the results obtained by two different electrophoretic methods, we can state the existence of two slightly different albumin-like proteins in the blood serum of the pike-perch.

The faster of the two paper electrophoretic albumin fractions, besides pure albumin, also contained a slowly moving component.

As in some other fishes (Kirsipuu, 1971, 1975a), the albumins of the pike-perch are lipoproteins. This became evident from paper electrophoretic analysis as well as from PAA gel electrophoresis (our unpublished data). Therefore, designating conventionally the fractions (or bands) discussed as albumins, we must keep in mind that biochemically they differ from the albumin of man and mammals.

The non-identified, hardly separable paper electrophoretic fraction «x» (Кирсипуу, 1964a) must factually be considered to be α_1-globulin as it consists of prealbumins mainly. This is a characteristic feature of α_1-globulin of man (Dugne et al., 1972) and of some fishes (Creyssel et al., 1964; Fine et al., 1963; Kirsipuu, 1975b, 1978).

The paper electrophoretic fraction designated by us as α_1-globulin (Кирсипуу, 1964a) is nearly of the same character. In addition to the common components with «x», it has some more insignificant ones (Fig. 2, N 1). Therefore, by paper electrophoresis these fractions can evidently be taken together.

The paper electrophoretic α_2-globulin consists mainly of two fast-moving components. In PAA gel they had the electrophoretic mobility

coinciding with that of albumins. The same phenomenon, the approximate coincidence of the electrophoretic mobility of albumin and an important α_2-globulin component at PAA gel electrophoresis was observed by us in the common carp (Kirsipuu, 1975b) and in the bream (Kirsipuu, 1978). As it is generally known, the separating role of the molecular size is very important at gel electrophoresis (this becomes evident also from the coinciding results of PAA gel electrophoresis and gel filtration of fish serum proteins, as indicated by H. Perrier et al., 1973), while by paper electrophoresis the main separating factor is the charge of molecules. Consequently, a similarity of molecule size and a difference of molecule charge of the fish serum albumins and most important α_2-globulin components can be expected. If so, the controversial quantitative changes of blood serum albumins and α_2-globulins in paper electrophoretic investigations (Кирсипуу, 1964b; Сорвачев, 1957, for example) should be discussed on the ground of osmoregulation.

No analogous component to human serum α_2-macroglobulin (α_2S) was detected in this fraction, while a significant slow band discovered in the α_1-fraction may be of this nature. The same is true of the bream (Kirsipuu, 1978).

The β-globulins, distinctly different at paper electrophoresis (where the difference of the dispositions was nearly 1 cm), were similar at PAA gel electrophoresis consisting of components of an intermediate moving speed. However, most of the components in β_1 and β_2 were slightly differing.

The β_o-component, connected with the ripening of roe (Kirsipuu, 1977) could not easily be differentiated. If comparing the PAA gel proteinograms of males and the female (Fig. 2), we can notice a slowly moving intense band in the female, while in males some faint bands can be detected at this disposition. In another investigation (our unpublished data) we could observe such an intense band (which was lipoprotein) at this disposition in all maturing females, while the absence of the band of similar intensity was always observed in males and immature females. So, analogous disposition of β_o at PAA gel electrophoregrams to the cyprinid fishes seems to be highly plausible, while a complete absence of this protein in males and immature females cannot be proved by our data. However, the analogous lipoprotein was surely found to be absent in males and immature females in our materials.

Some other sexual differences can also be detected. In the female specimen investigated the fast α_2-component was wholly absent while the slowest β_2-component was considerably intenser and an additional fast-moving β_1-component was detectable. In paper electrophoretic investigations on the pike-perch the increase of the relative amount of α_2-globulins was observed (Кирсипуу, 1964b, 1966; in that case β_1-globulin was added to α_2-globulin) and the appearance of β_1 was detected (Литвинова, 1968a, b) in females when roe was ripening. No significant increase in the α_1-fraction was observed by the authors, although in the female investigated an additional band, nearly coinciding with the sex-depending band at the whole serum PAA gel proteinogram was detected in the α_1-fraction. The additional band was relatively faint. Therefore, it seems that after treatment the β_o-component had changed its electrophoretic mobility. The appearance of some slowly moving intense β-components at repeated electrophoresis, which were evidently absent at the proteinogram of the whole serum, as well as the evidence of a changed mobility of some other components (Fig. 2) speak in favour of such supposition.

Obviously, in the pike-perch several protein components of the blood

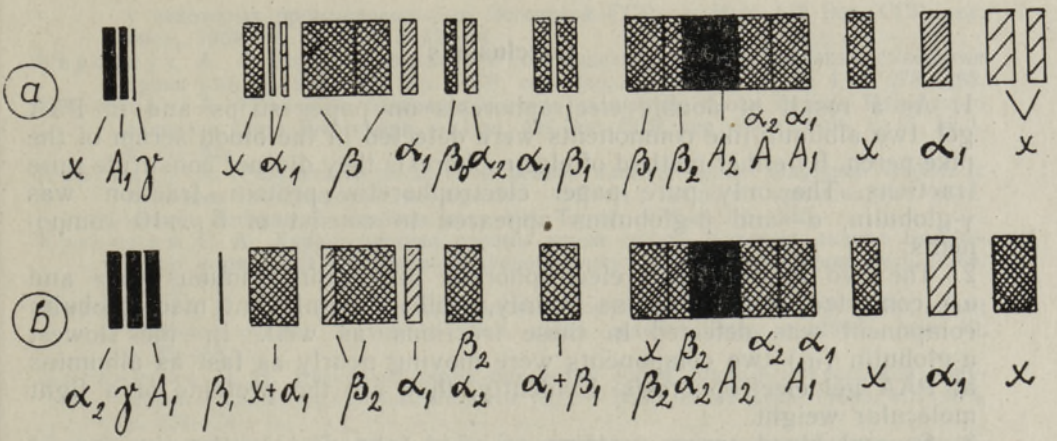


Fig. 3. The scheme of the distribution of paper electrophoretic blood serum protein fractions at PAA gel electrophoregram in the pike-perch; a) female, b) male.

serum take part in the ripening of roe, and their disposition and quantitative changes at proteinograms obtained by several electrophoretic techniques call for special investigations.

The paper electrophoretic γ-globulin fraction, consisting of a single protein, as in the carp and bream (Kirsipuu, 1975b, 1978), is of a high molecular weight and cannot be separately caught at PAA gel electrophoresis, since the components having an identical mobility at this technique, are present in the α-globulin fractions as well.

The distribution of the paper electrophoretic protein fractions of the blood serum of the pike-perch at PAA gel electrophoregram is presented in Fig. 3.

A surprising outcome was that many components, having coinciding mobility at PAA gel electrophoresis, are situated comparatively far in paper electrophoregrams. Such components were found in fractions α_1 and β_1, α_2 and β_2, «x» and γ, albumin and α_2. Evidently, they are the components of a similar molecular weight and different charge. We must keep in mind that by means of PAA gel electrophoresis their separation may fail.

Another significant result lies in the fact that after paper electrophoresis and extraction in sucrose solution, the electrophoretic mobility of some components was changed (as analogous bands were not detected at the PAA gel electrophoregrams of the whole serum) although denaturation and proteolysis were excluded. Most likely, the change of the electrophoretic mobility was caused by the change in the conformation and charge of the molecule as a result of eliminating the transported materials. An analogous situation is highly possible in natural conditions, too, and it must warn us to be careful when speaking about genetic stability of blood serum proteins on the grounds of the data obtained by means of PAA gel electrophoresis.

Different results in the paper electrophoretic separation of the pike-perch serum proteins can probably be due to such a lability of the blood serum protein system in this fish, as well.

Conclusions

1. As a result of double electrophoresis on paper strips and in PAA gel, two albumin-like components were detected in the blood serum of the pike-perch. By either method of electrophoresis they did not constitute pure fractions. The only pure paper electrophoretic protein fraction was γ-globulin, α- and β-globulins appeared to consist of 5...10 components.
2. The two fastest paper electrophoretic α-globulin fractions («x» and α_1) consisted of prealbumins mainly, while a significant macroglobulin component was detected in these fractions as well. In the slowest α-globulin (α_2) two components were moving nearly as fast as albumins at PAA gel electrophoresis. Evidently, they are the proteins of a light molecular weight.
3. Several blood serum proteins seem to take part in the ripening of roe in the pike-perch, as noticeable quantitative differences were detectable between PAA gel proteinograms of males and mature females. However, the results of the present work did not allow us to confirm the existence of qualitative differences in the blood serum proteins of sexes.
4. By repeated electrophoresis of paper electrophoretic blood serum proteins of the pike-perch, some components were detected which were absent at the PAA gel proteinograms of the whole serum. Consequently, by treatment the physico-chemical properties of some proteins are easily changed in this fish while an analogous possibility caused by changes of the physiological state of the fish must be taken into account in physiological and genetical investigations.

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KOHA (*LUCIOPERCA LUCIOPERCA*) VERESEERUMI VALKUDE KAHEKORDNE ELEKTROFOREES

Resümee

Koha vereseerumist paberelektroforeesi teel saadud valgufraktsioonide edasisel elektroforeesimisel polüakrüülamiidgeelis ilmnes, et ainsana on homogeenne γ-globuliini fraktsioon. Albumiinisarnaste valkude fraktsioonid, mida mõlema elektroforeesimeetodi abil avastati kaks, sisaldasid kumbki vähemalt üht suure molekulkaaluga komponenti. α- ja β-globuliinid koosnesid 5—10 komponendist. Kaks kiiremat paberelektroforeetilist α-globuliinide fraktsiooni olid väga sarnased, koosnedes peamiselt prealbumiinidest, kuid sisaldasid ka mõningaid makroglobuliine. Aeglasemate α-globuliinide hulgas leidus intensiivne albumiinisarnane komponent. Mitmes erinevas fraktsioonis ilmnes polüakrüülamiidgeelis sama liikuvusega komponente. Järelikult ei saa olla kindel, et kõik sel meetodil saadud fraktsioonid on homogeensed.

Erinevalt teistest seni uuritud kaladest näib kohal marja valmimisest osa võtvat enam kui üks vereseerumi valk, kuid ka kaladele iseloomulik marja valmimisega seotud lipoproteiin (β_o) oli tuvastatav. Ometi ei või käesoleva uurimuse põhjal kinnitada, et emaste ja isaste kohade vereseerumi valgusüsteemis on suguproduktide valmimise ajal selgeid kvalitatiivseid erinevusi.

Üksikute valgufraktsioonide edasisel elektroforeesimisel ilmnes mõningaid komponente, mis puudusid kogu seerumi polüakrüülamiidgeel-proteinogrammidel. Tõenäoliselt olid nende füüsikalis-keemilised omadused paberelektroforeetiliste fraktsioonide töötlemisel muutunud. Niisugune võimalus pole välistatud ka kala sisekeskkonna muutumise puhul eluprotsesside tulemusena, mida tuleks arvestada füsicloogilistes ja geneetilistes uurimustes.

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Toimetusse saabunud
14. X 1977

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ДВОЙНОЙ ЭЛЕКТРОФОРЕЗ БЕЛКОВ СЫВОРОТКИ КРОВИ СУДАКА (*LUCIOPERCA LUCIOPERCA*)

Резюме

При дальнейшем разделении бумажно-электрофоретических фракций белков сыворотки крови судака выяснилось, что гомогенной является лишь фракция γ-глобулинов. Фракции альбуминоподобных белков, которых при обоих методах электрофореза обнаружено две, содержали по крайней мере один компонент с высоким молекулярным весом. α- и β-глобулины содержали 5—10 компонентов. Две более быстрые фракции α-глобулинов оказались очень похожими, они состояли главным образом из преальбуминов, хотя и содержали ряд макроглобулинов. В более медленном α-глобулине найден интенсивный альбуминоподобный компонент. Во многих фракциях обнаружены компоненты, имеющие одинаковую подвижность в полиакриламидном (ПАА) геле. Поэтому мы не можем быть уверены, что все полученные с помощью электрофореза в ПАА геле фракции являются действительно гомогенными.

В отличие от других исследованных нами рыб у судака в созревании икры участвует, по-видимому, более чем один белок сыворотки крови, причем характерный для рыб связанный с созреванием икры липопротеин (β_o) также обнаружен. Однако по данному исследованию нельзя утверждать, что в белковой системе сыворотки крови самцов и самок судака во время созревания половых продуктов существуют явные качественные различия.

При дальнейшем электрофорезе отдельных бумажно-электрофоретических белковых фракций обнаружены некоторые компоненты, которые отсутствовали на ПАА-гелевых протеинограммах цельной сыворотки. Предполагается, что физико-химические свойства этих белков изменились в процессе обработки бумажно-электрофоретических фракций. Подобная возможность не исключена и при изменении внутренней среды рыбы в результате жизненных процессов, что необходимо учитывать в физиологических и генетических исследованиях рыб.

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