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DOUBLE ELECTROPHORESIS OF THE BLOOD PLASMA PROTEINS OF RAINBOW TROUT (*SALMO GAIRDNERI*)

The results of the paper electrophoretic fractionation of the blood serum or plasma proteins of salmonid fishes have been differently interpreted by different authors. Some of them have considered only the quickest fraction as albumin. It this case the relative amount of this protein in serum (plasma) is about 20 per cent (Drilhon, 1968; Остроумова, 1970; Клявсонс, 1970a, b). The others have considered two or three quick fractions to be albumins (in some cases not differentiated, obviously). According to them the relative amount of albumins is about 40 per cent or more in salmonids (Carbery, 1969; Meisner, Hickman, 1962; Snieszko et al., 1966; Кирсипуу, 1974; Новиков, Решетников, 1969). At the same time there are available data indicative of the «mixed» physico-chemical properties of the slowest albumin-like fraction in the rainbow trout, being albumin chemically — by the salting out, and α_1-globulin physically — by paper electrophoresis (Snieszko et al., 1966). By the way, the same has been shown concerning the albumin of cyprinid fishes (Белчева, Христов, 1972). Some authors completely deny the existence of albumin-like protein in the plasma of the rainbow trout (Perrier et al., 1974).

Besides that, some questions of sexual dimorphism in the blood serum (plasma) protein system in the rainbow trout are not clear so far, as the results of the paper electrophoretic investigations have been different (see Haider, 1970, and Кирсипуу, 1974) while the results obtained by means of different electrophoretic techniques seem not to be comparable (compare the authors mentioned and Thurston, 1967) as regards the albumin-like proteins, in particular. Therefore, it seemed to be useful to apply a method of double electrophoresis (Fine et al., 1963; Kirsipuu, 1975b, 1978a, b) to investigate the real heterogeneity and character of the protein fractions of blood plasma in the rainbow trout.

Material and methods

The heparinized plasmas of 5 sexually mature rainbow trouts from Aravuse trout-farm (North Estonia) were investigated in March, 1977. Two of them, one female (N 1) and one male (N 2), 5 years old were in excellent condition, ready to spawn (their gonads were in the V stage of development). Three of them (N 3 and 5 — males, N 6 — female) were 3 years old, poorly fed, gonads not ripe yet (the IV stage of development).

Plasma proteins were separated into fractions by means of paper electrophoresis, each fraction extracted in 40 per cent solution of sucrose

and subjected to electrophoresis in polyacrylamide gel as described in an earlier paper (Kirsipuu, 1975b).

Because of the small capacity of the electrophoretic chamber, not all fractional samples could be analyzed simultaneously and some of them were preserved frozen in the sucrose solution for up to 7 days. The second analysis in the polyacrylamide (PAA) gel of the samples analyzed immediately after extracting was carried out after 7 days in some cases, and it showed the stability of the samples under such conditions of preserving. The suitability of the samples preserved was concluded.

As it has been mentioned by several authors (McKenzie, Paim, 1969; Perrier et al., 1973; Груздев et al., 1972), no correct photographs or densitograms of PAA gel proteinograms can be obtained because of the blending of the faint fractions with the background or with more intense fractions (bands). Therefore it seemed to be reasonable to present schemes only, the more so since we shall not deal with quantitative relations here. To a certain extent, the relative intensity of bands is expressed in the schemes by the density of shading.

Results

By means of the paper electrophoresis we attained the separation of the blood plasma proteins of the rainbow trout into 7 fractions (earlier we gained up to 10 fractions in some cases — Кирсипуу, 1974) and interpreted the proteinograms as consisting of two albumin, two α-globulin, two β-globulin and one γ-globulin fractions (Fig. 1). The fastest of them

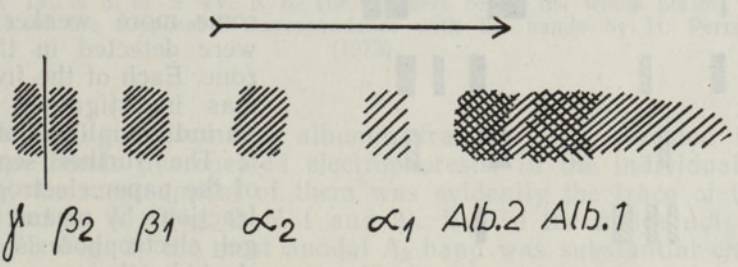
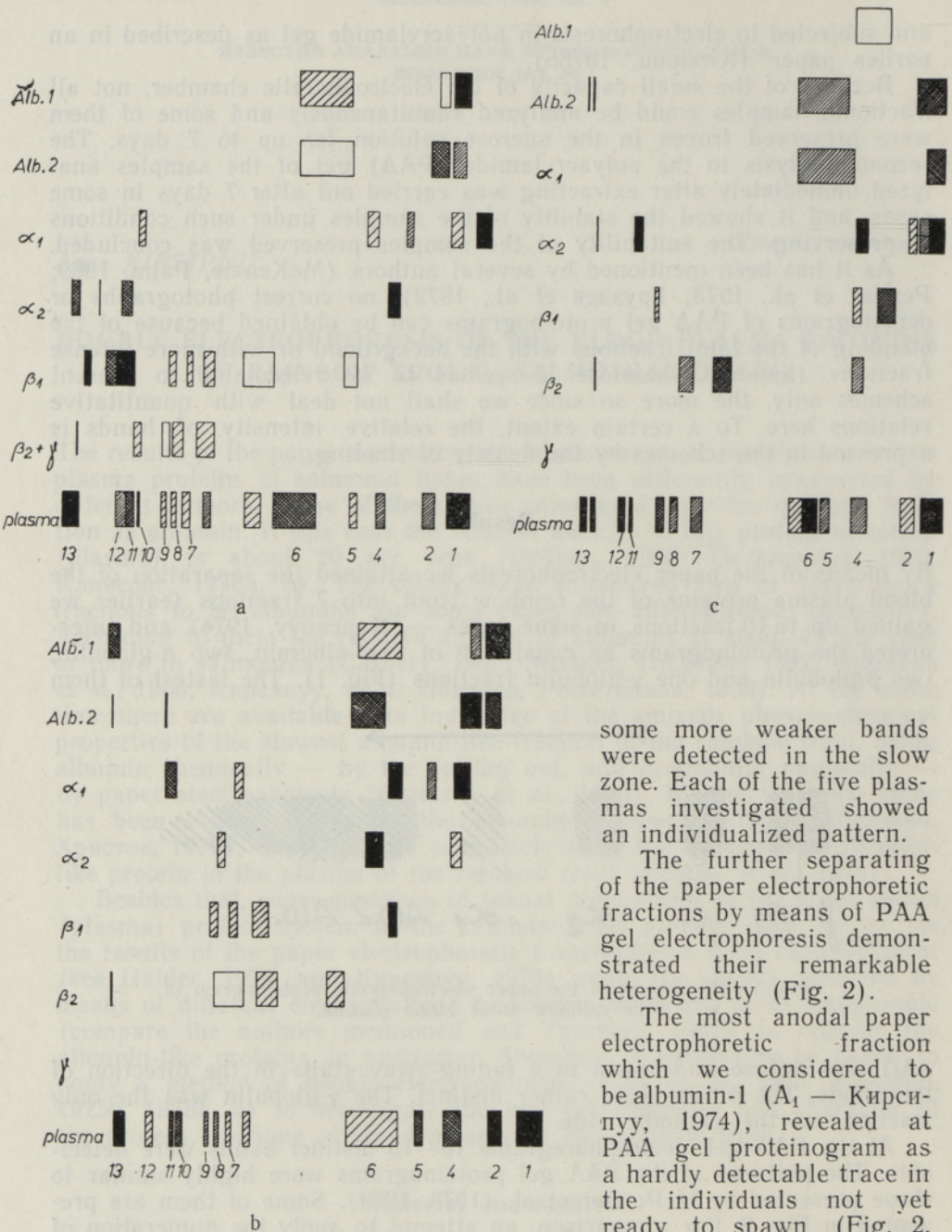


Fig. 1. The scheme of the paper electrophoretic proteinogram of the rainbow trout blood plasma.

(A_1) was diffuse and ended in a fading-away «tail» in the direction of the anode. The others were rather distinct. The γ-globulin was the only fraction on the cathodic side.

At the PAA gel electrophoregrams 13—15 distinct bands were detectable. The patterns of the PAA gel proteinograms were highly similar to those presented by H. Perrier et al. (1973, 1974). Some of them are presented in Fig. 2. For comparison, an attempt to apply the numeration of the bands by H. Perrier et al. (1973, 1974) is made, but the absolute correspondence cannot be guaranteed, naturally.

Individual variation in the bands 1 . . . 3 by the authors cited above was not observed in our examples. Moreover, band 3 (by H. Perrier et al., 1973, 1974) seemed to be absent at our proteinograms, as well as one of the bands 10 . . . 12 (most likely band 12). In our experiments band 13 was always divided into two, as it was observed by H. Perrier et al. (1973) if using 5.5 per cent PAA gel. Differences were found in the relative mobility and intensity of some bands (band 6, especially), while



some more weaker bands were detected in the slow zone. Each of the five plasmas investigated showed an individualized pattern.

The further separating of the paper electrophoretic fractions by means of PAA gel electrophoresis demonstrated their remarkable heterogeneity (Fig. 2).

The most anodal paper electrophoretic fraction which we considered to be albumin-1 (A_1 — Кирсипуу, 1974), revealed at PAA gel proteinogram as a hardly detectable trace in the individuals not yet ready to spawn (Fig. 2, N 3, 5 and 6). Its mobility somewhat differing in each individual, could not be identified with any band at the PAA gel proteinogram of the whole plasma. In the individuals ready to spawn, a distinct fast-moving band and besides it a trace, apparently transferred from the neighbouring fraction by paper electrophoresis, were observed (Fig. 2, N 1 and 2). Besides that, in these individuals a remarkable and very diffuse band was detectable. It evidently corresponded to band 6 by H. Perrier et al. (1973, 1974), although it was moving somewhat more quickly.

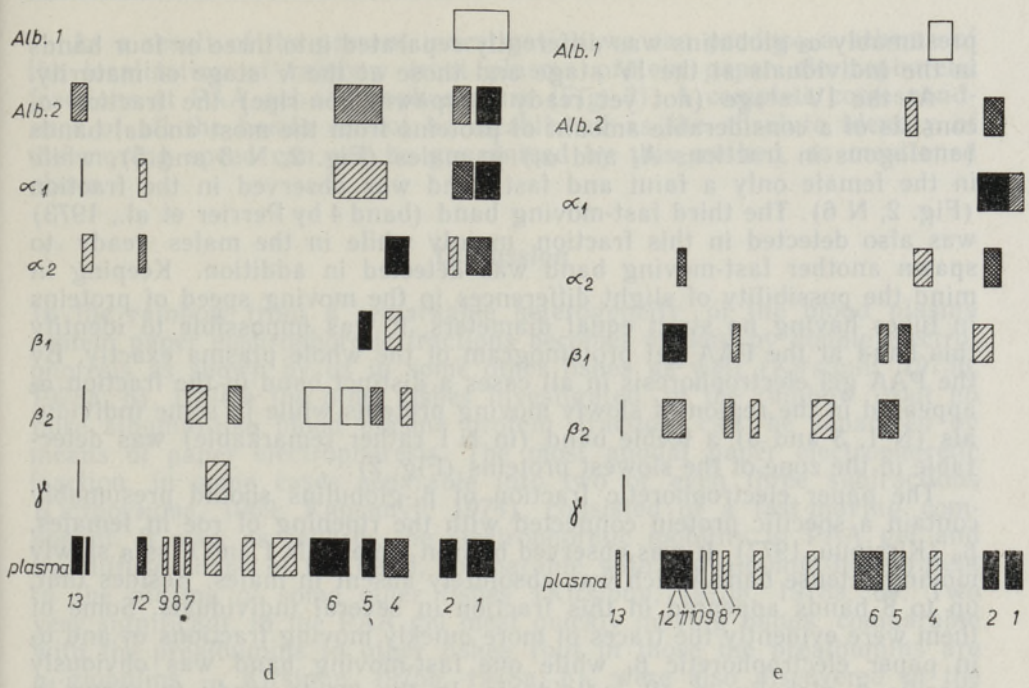


Fig. 2. Schemes of the PAA gel electrophoregrams of the paper electrophoretic blood plasma protein fractions in some rainbow trouts: a) ♀ V, N 1, b) ♂ V, N 2, c) ♂ IV, N 3, d) ♂ IV, N 5, e) ♀ IV, N 6. The numbers below the whole plasma proteinograms indicate the supposable correspondence with the bands by H. Perrier et al. (1973).

The following presumably albumin fraction (A_2) revealed in most cases three bands by repeated electrophoresis. In the individuals ready to spawn, the most anodal of them was evidently the trace of the analogous band in A_1 (Fig. 2, N 1 and 2). But in the males not yet ripe (N 3 and 5, Fig. 2) the most anodal A_2 band was substantial enough to be taken as a special band, especially because of lacking an analogous band in the A_1 paper-electrophoretic fraction. In the female not yet ripe (N 6, Fig. 2) the analogous band seemed to be absent in A_2, although it was present in the next, α_1-fraction, as well as in non-ripe males.

The next closely neighbouring band of this fraction was noticeable in the individuals ready to spawn while it was rather weak in other cases. A diffuse fast band, analogous to such in A_1 in ripened individuals, and, obviously, analogous to band 6 by H. Perrier et al. (1973, 1974), was always detectable. It was more significant in males. Besides that, in males a very slow band, fully absent in females, appeared in paper-electrophoretic fraction A_2.

Fraction α_1, very faint and sometimes even questionable at the paper electrophoretic proteinograms (Кирсипуу, 1974), after repeated electrophoresis in PAA gel revealed only the bands having identical mobility with those in A_2 in the individuals not yet ripe (Fig. 2). In this case the bands were too considerable to be taken as traces from the neighbouring fractions. In the individuals ready to spawn two additional fast bands, analogous to those in α_2 in non-ripe individuals (bands 4 and 5) were observed, and so were one or two slowly moving bands (Fig. 2).

By PAA gel electrophoresis the paper electrophoretic fraction of

presumably α_2-globulins was differently separated into three or four bands in the individuals at the IV stage and those at the V stage of maturity.

At the IV stage (not yet ready to spawn, non-ripe) the fraction α_2 consists of a considerable amount of proteins from the most anodal bands (analogous in fractions A_2 and α_1) in males (Fig. 2, N 3 and 5), while in the female only a faint and fast band was observed in the fraction (Fig. 2, N 6). The third fast-moving band (band 4 by Perrier et al., 1973) was also detected in this fraction mainly while in the males ready to spawn another fast-moving band was detected in addition. Keeping in mind the possibility of slight differences in the moving speed of proteins in tubes having no strict equal diameters, it was impossible to identify this band at the PAA gel proteinogram of the whole plasma exactly. By the PAA gel electrophoresis in all cases a distinct band in the fraction α_2 appeared in the region of slowly moving proteins while in some individuals (N 1, 3 and 5) a feeble band (in N 1 rather remarkable) was detectable in the zone of the slowest proteins (Fig. 2).

The paper electrophoretic fraction of β_1-globulins should presumably contain a specific protein connected with the ripening of roe in females, β_o (Kirsipuu, 1977). It was observed both in females N 1 and 6 as a slowly moving intense band which was absolutely absent in males. Besides that, up to 8 bands appeared in this fraction in several individuals. Some of them were evidently the traces of more quickly moving fractions α_1 and α_2 in paper electrophoretic β_1 while one fast-moving band was obviously specific β_1-globulin in all individuals. In the trouts ready to spawn a remarkable part of slowly moving proteins localized in fraction β_1.

Fraction β_2 also consists of some slowly moving proteins, and, besides that, in this fraction there appeared an intermediate band, obviously corresponding to band 6 by H. Perrier et al. (1973) which was «lost» by gel filtration in their experiments. In the individuals not yet ready to spawn a detectable trace from faster fractions (α_2) could be observed and, evidently, a part of band 5.

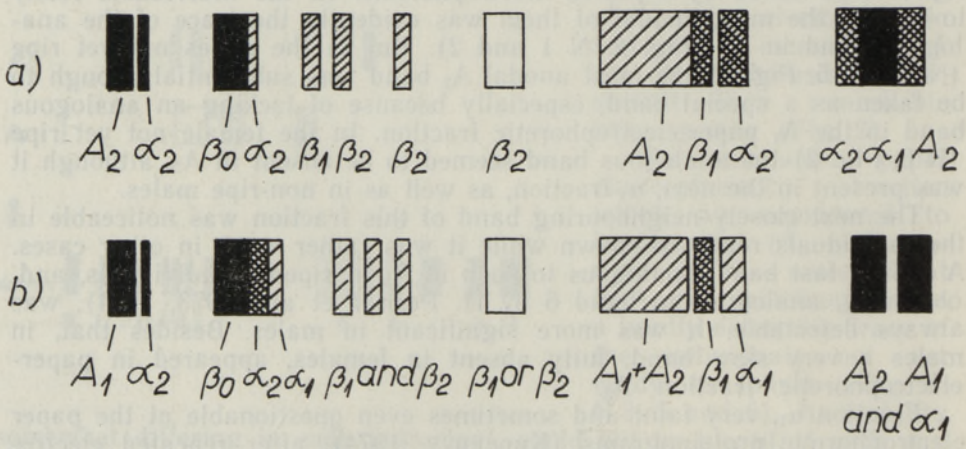


Fig. 3. The scheme of the distribution of paper electrophoretic blood plasma protein fractions at PAA gel electrophoregram in the rainbow trout: a) not ready to spawn, b) ready to spawn.

γ-globulin fraction, though remarkable at paper electrophoregrams, was detectable as a feeble band of the slowest mobility only; in some cases it did not reveal any band in PAA gel.

As a result of the present investigation we can produce a scheme of the localization of rainbow trout plasma protein paper electrophoretic fractions at PAA gel electrophoregram (Fig. 3). A complete correspondence of all the bands cannot be established as the absolute identity of the moving speed can not be guaranteed by this method, as mentioned above.

Discussion

In the rainbow trout a remarkable heterogeneity of the blood plasma protein paper electrophoretic fractions becomes evident by double electrophoresis as shown by us in some other fishes as well (Kirsipuu, 1975b, 1978a, b). Unlike the other fishes investigated, in the rainbow trout no pure albumin-like blood plasma protein fraction can be separated by means of paper electrophoresis. The most anodal paper electrophoretic fraction, in some cases separable into two or even three subfractions (Остроумова, 1969; Кирсипуу, 1974), consisted of a fast-moving component. It was, as regards the electrophoretic mobility in PAA gel and the diffuse shape of the band, comparable with the albumin-like protein in the plasma of some other fishes (Kirsipuu, 1975b, 1978a, b). Two very significant, in the PAA gel most quickly moving bands, comparable with the prealbumins in other fishes (but in those the prealbumins are α_1-globulins — Kirsipuu, 1975b, 1978a, b), were also discovered in the fraction. Besides that, the existence of some components of very light molecule weight (moving exceptionally quickly by PAA gel electrophoresis), in this paper electrophoretic fraction, especially in the subfraction A_1, is very likely, as the completely distinguishable paper electrophoretic A_1 fraction revealed no bands by repeated electrophoresis in PAA gel in non-ripe individuals. By H. Perrier et al. (1973), such very quick components were observed in the rainbow trout plasma if the running time by PAA gel electrophoresis was shortened.

On the other hand, some components in the fractions β_1 and α_2 had nearly the same mobility in PAA gel as the albumin-like band. Therefore, evidently, the albumin-like plasma protein of the rainbow trout can never be gained as a pure fraction by means of unifold electrophoresis.

Taking into account the mixed character of the fraction discussed, we can suppose that the real amount of the albumin-like protein in the blood plasma (serum) of the rainbow trout is much lower than indicated in the paper electrophoretic investigations (Остроумова, 1969; Кирсипуу, 1974). It is in harmony with the general opinion, according to which in the lower animals the relative amount of blood plasma (serum) albumin is low, since the Salmonidae are ancient (lower) fishes (Kuhn, 1967; Берг, 1940).

As in some fishes investigated before (Kirsipuu, 1971, 1975a, 1978b), the albumin-like protein in the rainbow trout is a lipoprotein as well: the homologous fraction (or band) was stained as lipoprotein both by paper electrophoresis (Кирсипуу, 1974) and by PAA gel electrophoresis (Perrier et al., 1974).

The other fractions of the blood plasma proteins of the rainbow trout are, in general, comparable with those in other fishes (Kirsipuu, 1975, 1978a, b). α_1-globulin consists of prealbumins, mainly. The same is shown in man and in many fishes (Drilhon, Fine, 1960; Fine et al., 1963; Creyssel et al., 1964; Kirsipuu, 1975b; Тодоров, 1963; Лукьяненко et al., 1967). α_2-globulin consists of an important fast component (α_2F), having the electrophoretic mobility in PAA gel slightly quicker than albumin, a

slowly moving component analogous to α_2S and of a very slow component. The fractions of β-globulins include postalbumins (presumably transferrins, if supposing analogy to the carp and bream — Creyssel et al., 1964; Kirsipuu, 1978a, see also Drilhon, 1968), intermediate components (presumably haptoglobins amongst them) and slow components, amongst them the specific for maturing females β_o. γ-globulin is supposedly consisting of a single very slow (high molecular weight) and negligible component, as it was observed in the pike-perch, too (Kirsipuu, 1978b).

Unlike to R. Thurston (1967), two sex-specific proteins in the blood plasma of the rainbow trout were discovered by us. In females it was the lipoprotein β_o, characteristic of the ripening roe (Kirsipuu, 1977), in the β_1-globulin fraction. In males it was a protein of high molecular weight in the albumin-like paper electrophoretic fraction. As immature individuals were not investigated, we cannot find out if it is specific for males at all or it is connected with the ripening of milt only.

Some peculiarities connected with the ripening of sexual products become evident. In the individuals ready to spawn, a fastening of several components (albumins and prealbumins first and foremost) by paper electrophoresis was observable, while the stability of their moving speed by PAA gel electrophoresis was evident. Keeping in mind that by paper electrophoresis the charge of the molecule is the main separating factor while by gel electrophoresis the size of molecules also plays an important role (Fine et al., 1963), we must conclude that at the last stage of maturity of gonads a change in the charge of molecules of these proteins takes place. The real cause of this phenomenon may be a change in the transport functions of molecules. Evidently, such an effect calls forth a rise in the amount of the most anodal blood serum protein fraction in the female rainbow trouts before spawning (Thurston, 1967; Остроумова, 1969). This is in contradiction with the data obtained concerning other fishes, in which the remarkable diminishing of the most anodal fraction by ripening of gonads (especially in females) was observed (Кирсипуу, 1964; Головко, 1964; Литвинова, 1970; Einszporn-Orecka, 1970, and many others).

Besides the peculiarities mentioned above, the formation of some additional bands (the most anodal α_1 in the female N 1) and the disappearance (the most anodal β_1 in ripe individuals) or noticeably weakening of some bands (slow β_2 bands in ripe individuals) seemed to be connected with the definitely ripening of sexual products. But the scanty material does not allow us to make any final conclusions on the subject.

As some bands evidently changed their mobility in PAA gel after separating by paper electrophoresis (Fig. 2, N 2 and 3, for example) and some bands of paper electrophoretic fractions seemed to be absent at the proteinograms of the whole plasma in PAA gel (Fig. 2, N 1, 3 and 5, for example), we may conclude what was observed by us in the pike-perch (Kirsipuu, 1978b): some blood plasma proteins of the fish investigated are highly unstable. Evidently, they are easily changing their conformation and charge. The possibility of mutual transition of mono- and polymer forms of proteins (see Drilhon et al., 1968) must be taken into account as well.

If keeping in mind the «fastening» of some blood plasma proteins by paper electrophoresis, when the gonads are finally ripening, the mentioned instability of some components and the significant complexity of paper electrophoretic plasma protein fractions, we must assume that

the physiological changes in the plasma protein paper electrophoretic fractions of the rainbow trout cannot be compared with those in some other fishes, in which the blood plasma (serum) proteins are more stable (in cyprinids, especially, — Kirsipuu, 1975b, 1978a). Because of the complexity of many fractions (and bands as well) the real cause of physiological changes in the blood plasma protein system is scarcely discoverable by unifold electrophoresis method in this fish.

Conclusions

1. In the rainbow trout the composition of paper electrophoretic blood plasma protein fraction and the disposition of these components at PAA gel proteinograms is, in general lines, similar to those in other fishes investigated by us before.
2. By the method of double electrophoresis in the blood plasma of the rainbow trout an albumin-like protein can be detected, which in analogous to that in other fishes and possesses similar electrophoretic mobility and shape of the band, and is lipoprotein. But this protein cannot be determined by unifold electrophoresis, as some other blood plasma proteins have nearly the same electrophoretic mobility by both electrophoretic methods used here.
3. The existence of γ-globulin in the blood plasma of the rainbow trout is questionable.
4. In the blood plasma of the maturing females of the rainbow trout a specific slowly moving lipoprotein, β_0, appears and in the males a sex-specific protein of high molecular weight in the albumin-2 fraction is detectable. In the fish ready to spawn, both in males and females, a «fastening» (a transfer into more fast fractions) of some proteins by paper electrophoresis becomes evident, but not by PAA gel electrophoresis. This is, apparently, a result of the change in the charge of the protein molecules.
5. Some blood plasma proteins of the rainbow trout evidently easily change the conformation and charge of molecule and therefore also change their disposition at PAA gel proteinograms after treatment of plasma. Such a possibility must be taken into account at the change of the physiological state of the fish, too.
6. By the reasons mentioned in clauses 4 and 5, the physiological changes in the paper electrophoretic fractions of blood plasma proteins in the rainbow trout are not comparable with those in some other fishes.

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VIKERFORELLI (*SALMO GAIRDNERI*) VEREPLASMA VALKUDE KAHEKORDNE ELEKTROFOREES

Viie suguküpse vikerforelli vereplasmast paberelektroforeesi teel saadud valgufraktsioonide elektroforeesimisel polüakrüülamiidgeelis (PAA-geel) ilmnes paberelektroforeetiliste fraktsioonide suur heterogeensus, kuid nende jaotumine komponentideks ja viimaste paiknemine PAA-geeli elektroforegrammidel oli üldjoontes samasugune kui varem uuritud kalaliikidel.

Albumiinisarnast valku ei ole võimalik eraldada puhta fraktsioonina kummagi elektroforeesimeetodi abil, sest mõlemal juhul oli sama liikuvusega ka mõni teine vereplasma valk. Järelikult on vikerforelli vereplasmas albumiinisarnase valgu hulk märksa väiksem, kui seni ühekordse elektroforeesi tulemuste alusel arvati.

Nii nagu teistelgi kaladel, ilmub emaste vikerforellide verre marja valmimise ajal spetsiifiline lipoproteiin. Kuid erinevalt seni uuritud liikidest leidus ka isaste vikerforellide vereplasmas soospetsiifilist valku, mis oli (otsustades elektroforeesi põhjal PAA-geelis) suure molekulkaaluga, kuigi liikus paberelektroforeesil albumiinisarnases (A_2) fraktsioonis.

Paberelektroforeetiline γ-globuliinide fraktsioon jäi PAA-geelis enamasti tühjaks, mistõttu γ-globuliinide olemasolu vikerforelli veres on kaheldav.

Kahekordse elektroforeesi tulemusena selgus veel, et vikerforelli gonaadide lõplikul valmimisel lähevad mõned vereseerumi valgud paberelektroforeesil üle kiiremasse fraktsiooni, kuid elektroforeesil PAA-geelis nende liikuvus ei muutu. Arvatavasti on selle põhjuseks valgumolekulide laengu muutumine seoses transportülesannete muutumisega.

Osa paberelektroforeetilistest valgufraktsioonidest PAA-geelelektroforeesi abil eraldatud komponente ei olnud identifitseeritavad ühegi ribaga kogu plasma PAA-geel-proteinogrammil. Oletatavasti muudavad need valgud vikerforelli plasma töötlemisel sahharoosi lahuses kergesti oma laengut ja konformatsiooni. Võimalik, et sellised muutused toimuvad ka füsioloogilistes protsessides kala organismis. Sel juhul on vikerforelli vereplasma valgusüsteemi füsioloogilise muutlikkuse uurimine elektroforeesimeetodil raske.

Аарe КИРСИПУУ

ДВОЙНОЙ ЭЛЕКТРОФОРЕЗ БЕЛКОВ ПЛАЗМЫ КРОВИ РАДУЖНОЙ ФОРЕЛИ (*SALMO GAIRDNERI*)

Белки плазмы крови пяти половозрелых особей радужной форели разделены на фракции с помощью электрофореза на бумаге, а затем каждая фракция подвергнута повторному электрофорезу в полиакриламидном (ПАА) геле. Обнаружена большая гетерогенность бумажноэлектрофоретических фракций; их разделение на компоненты и расположение последних на ПАА-гелевых электрофореграммах были в общих чертах похожи на таковые у карпа, леща и судака.

Ни тот, ни другой метод электрофореза не дал возможности получить чистую фракцию альбуминоподобного белка, так как в обоих случаях вместе с ней двигался и еще какой-нибудь белок плазмы. Следовательно, истинное содержание альбумино-

подобного белка в плазме крови радужной форели значительно меньше, чем считалось до сих пор по данным однократного электрофореза.

Как у других рыб, так и у радужной форели во время созревания икры в крови у самок появляется липопротеин. Но в отличие от рыб, исследованных нами ранее, у радужной форели в крови самцов обнаружен также специфический для этого пола белок, который, судя по данным электрофореза в ПАА геле, отличается большим молекулярным весом, хотя при электрофорезе на бумаге он двигался с фракцией альбуминоподобных белков (A_2).

Бумажноэлектрофоретическая фракция γ-глобулинов в большинстве случаев не выявила никаких полос в ПАА геле, поэтому присутствие γ-глобулинов, аналогичных таковым у других рыб, в крови у радужной форели сомнительно.

В результате двойного электрофореза выявлено также, что у радужной форели при окончательном созревании гонад некоторые плазменные белки при электрофорезе на бумаге переходят в более быстрые фракции, а при электрофорезе в ПАА геле их подвижность не изменяется. Причиной этого является, по-видимому, изменение заряда белковых молекул в результате изменения их транспортной функции.

Некоторые компоненты, выделенные из бумажноэлектрофоретических фракций с помощью электрофореза в ПАА геле, не удалось идентифицировать ни одной полоской, полученной на ПАА-гелевой протеинограмме цельной плазмы. Видимо, эти белки легко изменяют конформацию и заряд при обработке плазмы в растворе сахарозы. Не исключено, что подобные изменения легко происходят и в организме во время физиологического изменения внутренней среды.