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RESULTS OF AN ELECTROPHORETIC INVESTIGATION OF BLOOD SERUM LIPO- AND GLYCOPROTEIDS IN SOME CYPRINID FISHES

For ascertaining the role of blood serum proteins in metabolism, it is necessary to elucidate their relations with other fundamental substances in metabolism — fats and carbohydrates. Important links in those relations are lipo- and glycoproteids of blood serum. Investigations on their dependence upon the physiological state of an organism are significant for physiologists for understanding the mechanisms of metabolic processes.

As justly indicated by N. Kulikova (Куликова, 1967), the blood serum lipoproteids of fishes have been rather poorly investigated up to the present time, and the same can be said of the serum glycoproteids. Therefore, in our opinion, the investigation of these components in the blood serum of fishes, apart from researches into their blood serum proteins, is a problem of prime importance.

Materials and methods

The present work is based on 136 lipo- and 134 glycoproteidograms of blood serum of bream (*Abramis brama*) from lake Võrtsjärv and from some minor Estonian lakes, and on some lipo- and glycoproteidograms of roach (*Rutilus rutilus*), tench (*Tinca tinca*), crucian (*Carassius carassius*), ide (*Leuciscus idus*) and *Aspius aspius*. For an identification of lipo- and glycoproteid fractions, the electrophoregrams of the blood serum proteins were prepared simultaneously.

Blood was collected by heart punction after opening the body cavity. After clotting at room temperature, the serum was gathered by means of a micropipette.

For carrying out the electrophoresis of lipo- and glycoproteids, 0.05 ml of serum was drifted to a filter paper strip preliminarily moistened with buffer. The Leningrad chromatographic paper "Б" strips of 2×40 cm and Na-veronal buffer with pH 8.6 and μ 0.01 were used.

Electrophoresis was carried out in vertical chamber at a temperature of +5 °C during 12 hours. The current was 0.40—0.50 mA/cm, and the tension 400 V. After electrophoresis, the strips were dried at +115°. Lipoproteids were stained by Swan (Тодоров, 1963) and glycoproteids by Kõiw and Grönvall (Тодоров, 1963). The fractions were estimated visually, as well as the relative intensity of fractions.

Results and discussion

In the lipoproteidograms of the breams investigated, we could find 1 or 2 fractions (Fig. 1). In all specimens, irrespective of sex or season when analyzed, a fraction was distinguished, which migrated with albumins. The

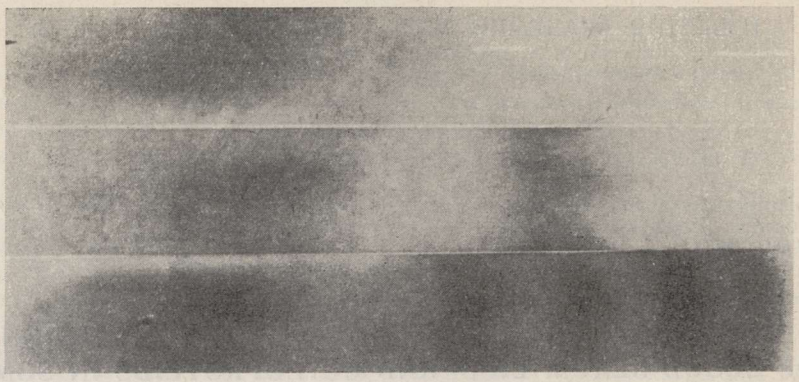

Fig. 1. Lipoproteidogram of the blood serum of the bream: *a* — male, *b* — female, *c* — proteidogram.

density of that fraction was comparatively stable. In males and immature females, as well as in mature females in summer (July-September), no traces whatever of other fractions were found. But in October, in the blood of mature females, another fraction appeared, which migrated with α_2-globulins. That fraction grew more and more intensive until May and disappeared at the end of June, roughly one month after spawning. Undoubtedly, that fraction is connected with the transport of lipoproteids for egg formation. The appearance of the lipoproteids in the α_2-globulin fraction also calls forth an augmentation of the protein part of that fraction at the time of the ripening of hard roe, as shown by us in earlier works (Кирсипуу, 1964a, 1964b, 1965; Кирсипуу, Пиху, 1965). The augmentation of some fractions or the appearance of new fractions in connection with the appearance of lipoproteids in the blood of fishes, in connection with the ripening of hard roe, has also been described by Van Dzu-sun and Van Dzin-bao (Ван Цзу-сюн, Ван Цзинь-бао, 1964) and N. Kulikova (Куликова, 1967).

Analogous lipoproteidograms and their sexual differences were observed by us in the other cyprinid fishes mentioned above.

The glycoproteidograms of the blood serum of cyprinid fishes acquired colour weakly, which points to the low glycoproteid content in the blood serum of those fishes. Four fractions were found migrating respectively with albumins α_1-, α_2- and β-globulins (Fig. 2). γ-glycoproteid is obviously absent. Sometimes we noticed a loose band at the start line, but it was probably a trace of a weak haemolysis which could not be ascertained by visual methods. In some cases, an absence of the β-glycoproteids was observed. It is possible that the weak fraction is sometimes not separable from the background or from the trace left by faster fractions. The general appearance of the glycoproteidograms as well as that of lipoproteidograms was identical in all the cyprinid fishes investigated.

The relatively abundant material permits us to make some intimation on the possibility of the seasonal quantitative changes in the glycoproteidograms of the blood serum of the bream.

The most important is the fraction of α_1-glycoproteids. The density of that fraction is comparatively stable, and therefore the density of the other fractions was estimated in respect to that one. As a result of visual assessment, it became evident that the density of the fraction which migrates with albumins is greater in May than in the other months, being also greater in males than in females. The relative density of

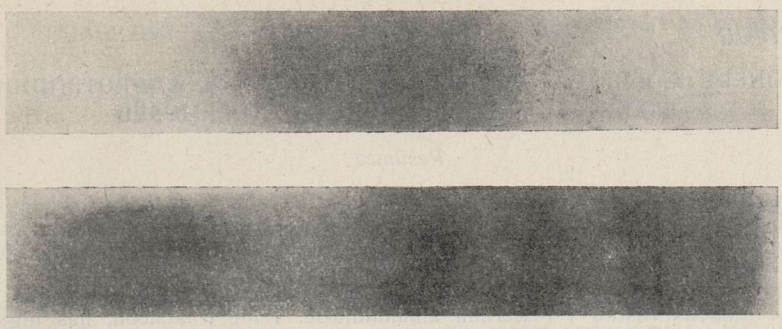

Fig. 2. Glycoproteidogram of the blood serum of the bream: *a* — glycoproteidogram, *b* — proteidogram.

α_2-glycoproteids is lower in winter. These preliminary results must undoubtedly be checked by means of biochemical analysis. But here one must keep in mind that the glycoproteidograms must be photographed and the colorimetric analyses must be effected immediately after staining and drying, since at storage the background gets intensively red again.

Conclusions

1. In the blood serum of bream and some other cyprinid fishes only one permanent lipoproteid fraction was observed, which migrates by paper electrophoresis with the albumins of blood serum. In connection with the ripening of hard roe in the blood serum of females, another lipoproteid fraction appears, which migrates with α_2-globulins.
2. The blood serum glycoproteids of these cyprinid fishes can be separated into 4 fractions by means of paper electrophoresis. These fractions migrate respectively with albumins, α_1-, α_2- and β-globulins. γ-glycoproteids seem to be absent in those fishes. It is possible that the amount of glycoproteids in some fractions is subjected to seasonal changes.
3. Species specifity of lipo- and glycoproteidograms in the cyprinid fishes investigated was not ascertained.

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MÕNEDE KARPLASTE VERESEERUMI LIPO- JA GLÜKOPROTEIIDIDE ELEKTROFOREETILISE UURIMISE TULEMUSED

Resümee

Paberelektroforeesi teel valmistati 136 lipo- ja 134 glükoproteidogrammi latika ning mõned lipo- ja glükoproteidogrammid särje, linaski, kogre, säina ja tõugja vereseerumist. Lipoproteidogrammid värviti Swani meetodil, glükoproteidogrammid Kõiwu ja Grönvalli järgi. Rõõbiti sellega elektroforeesiti vereseerumi valgud.

Latika vereseerumis esineb alaliselt üks lipoproteiidide fraktsioon, mille elektroforeetiline liikuvus vastab vereseerumi albumiinidele. Teine fraktsioon, mis liigub koos α_2-globuliinidega, esineb ainult suguküpsete emaste veres marja valmimise perioodil. Samasugune oli ka kõigi teiste ülalnimetatud karplaste vereseerumi pilt.

Glükoproteiidid jaotusid kõigil meie poolt uuritud karplastel neljaks fraktsiooniks, millede elektroforeetiline liikuvus vastas albumiinidele ning α_1-, α_2- ja β-globuliinidele. γ-glükoproteiid näib nende kalade vereseerumis puuduvat. Latika glükoproteidogrammide fraktsioonide suhtelise tugevuse hindamisel silma järgi ilmnes, et glükoproteiidide hulk mõnedes fraktsioonides allub tõenäoliselt sesoonsetele muutustele.

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РЕЗУЛЬТАТЫ ЭЛЕКТРОФОРЕТИЧЕСКОГО ИССЛЕДОВАНИЯ ЛИПО- И ГЛИКОПРОТЕИДОВ СЫВОРОТКИ КРОВИ НЕКОТОРЫХ КАРПОВЫХ РЫБ

Резюме

Методом электрофореза на бумаге было получено 136 липо- и 134 гликопротеидограмм сыворотки крови леща и несколько липо- и гликопротеидограмм сыворотки крови плотвы, линя, карася, язя и жереха. Липопротеидограммы окрашивали методом Свана, гликопротеидограммы — по Кыйв и Гренваль. Параллельно был проведен электрофорез белков сыворотки крови. Постоянно в сыворотке крови леща наблюдается одна фракция липопротеидов, электрофоретическая подвижность которой соответствует сывороточным альбуминам. Другая фракция, движущаяся вместе с α_2-глобулинами, появляется в крови половозрелых самок в период созревания икры. Такое явление обнаружено также у всех названных выше карповых рыб.

У всех изученных нами карповых гликопротеиды разделялись на четыре фракции, электрофоретическая подвижность которых соответствовала альбуминам, α_1-, α_2- и β-глобулинам. γ-Гликопротеид, по-видимому, отсутствует в крови этих рыб. При визуальной оценке относительной интенсивности фракций на гликопротеидограммах леща выяснилось, что количество гликопротеидов в некоторых фракциях, вероятно, подвергается сезонным колебаниям.

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