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FURTHER SEPARATION OF PAPER ELECTROPHORETIC BLOOD SERUM PROTEIN FRACTIONS OF CARP (*CYPRINUS CARPIO*) BY DISC ELECTROPHORESIS IN POLYACRYLAMIDE GEL

By means of disc electrophoresis in polyacrylamide (PAA) gel, 12—18 and, in some cases, even more than 20 protein fractions have been found in the blood serum of fishes (Salibian, 1965; Salibian, 1967; Thurston, 1967; Груздев et al., 1972; Лукьяненко et al., 1967; Лукьяненко et al., 1968; Лукьяненко, Попов, 1971; Седов, Лукьяненко, 1969; Яаска, Кирсипуу, 1971). But the origin of the fractions and their identity with the fractions obtained by other techniques are not clear so far. Therefore, the designation of the fractions obtained by means of PAA gel electrophoresis by different investigators is very different.

The present paper represents the results of an attempt to identify the paper electrophoretic blood serum protein fractions as those obtained by means of disc electrophoresis in PAA gel. Applying the idea of A. Drilhon and her collaborators (Drilhon et al., 1962), the eluates of paper-electrophoretic globulin fractions were analyzed by means of PAA gel electrophoresis.

Materials and methods

The sera of three 2-year-old common carps, two of them immature females (No. 4 and 5) and one mature male (No. 8) were separated by paper electrophoresis in vertical chamber into five fractions: albumins, α_1-, α_2-, β- and γ-globulins. Electrophoresis was carried out during 20 h* with Na-veronal buffer (pH 8.6) at a temperature of +5 °C. The voltage was 400 V and the current was 0,4 mA/cm. On each of the 12 strips 2×40 cm 0.03 ml of serum was dropped. After electrophoresis some strips were quickly dried at a temperature of +110° and stained with bromphenol blue. Three electrophoregrams were maintained in a wet chamber so long. Then, in comparison with stained electrophoregrams, the areas of globulin fractions were determined at them and the central part of each fraction was cut off. The wet filter paper pieces were set each in a test tube into 0.03 ml of 40 per cent solution of sucrose for an hour, in order to extract proteins from the paper. Then the protein extract in sucrose (we succeeded in obtaining about 0.02 ml) was subjected to electrophoresis in PAA gel according to the instruction by the gel electrophoresis complex of "Reanal" (Hungarian production) during 2.5 hours at a room temperature (24°) by the voltage of 320 V and total current of 27—28 mA (2.3 mA to each tube). Prescription is the following.

3.5 ml of solution: 48 ml HCl 1N, 36.6 g tris-aminomethane, 0.23 ml N,N,N′,N′-tetramethylamidin, aq. dest. ad. 100 ml;

* During this time albumins ran away from the strip, but we strove for a more distinct separation of globulin fractions.

7.0 ml of solution: 28 g acrylamide, 0.735 g N,N′-methylenbis-acrylamide, aq. dest. ad. 100 ml;
3.5 ml aq. dest.;
14.0 ml of solution: 0.140 g of ammonium persulphate in 100 ml aq. dest.
The buffer used: 1.2 g of tris-aminomethane, 5.76 g of glycine in 2000 ml of aq. dest.

Results and discussion

After staining with amidoblack 10B we could observe certain fractions at each PAA electrophoregram. The results of disc-electrophoretic fractionation of our materials are presented in Figs. 1 and 2. The fractions at the PAA electrophoregrams of the serum of carp No. 8 were hardly noticeable, and, therefore we cannot present the photographs of these electrophoregrams.

Obtained by means of paper electrophoresis, the fraction of α_1-globulins revealed after PAA gel electrophoresis two fractions: one of them moved with the velocity of prealbumin, while the other appeared to move with the velocity ranging between that of prealbumin and albumin. As at the PAA electrophoregram of the whole serum not a fraction was detected in this place, we assume that some components move differently in the mixture and in the pure solution, i. e. several components have their effect on the moving velocity of the others.

The paper electrophoretic fraction of α_2-globulins was divided into three fractions. One of them moved a little more quickly than albumins, and it is comparable with α_2F at starch gel electrophoresis (Drilhon et al., 1962).

The other two were markedly slower. One of them appeared in the zone of transferrins (Kirsipuu et al., 1972), another one should be taken as α_2S.

The most heterogeneous was the paper-electrophoretic fraction of β-globuiins. Moreover, this fraction was divided differently in different specimens. In carp No. 4 only one very clear fraction appeared in the zone next to albumins, whereas in carp No. 8 four very weak, slowly moving fractions appeared. Seven fractions were revealed there after the run of paper-electrophoretic β-globulins in PAA gel in carp No. 5. The quickest of them moved with the velocity of albumins, the most important of them seemed to be postalbumin. The others were rather weak and moved slowly. The comparatively important paper-electrophoretic fraction of γ-globulins revealed at PAA electrophoregrams only as some very weak, slowly moving fractions.

It is evident that the fractions obtained by paper-electrophoresis are not pure: in each separation by PAA gel electrophoresis we could observe some traces of the neighbouring fractions. So, the traces of the slow-moving α_2-lipoproteid were always detectable at the PAA gel electrophoregrams of α_1-globulins and at those of β-globulins as well.

As a matter of fact, due to the significant dilution of protein in the process of extraction, by means of this method we could observe only the most important fractions at the PAA gel electrophoregrams. To discover some less important fractions, the dropping of some more serum on the paper strips is needed. But in such a case it may happen, that the separation of fractions deteriorates.

As a result, we can give a provisional scheme of the distribution of the paper-electrophoretic fractions of the carp's serum by disc electrophoresis in PAA gel, as presented in Fig. 3. In comparing this scheme with the distribution of the blood serum proteins of carp by starch gel electrophoresis, a certain similarity can be detected (Drilhon, 1960;

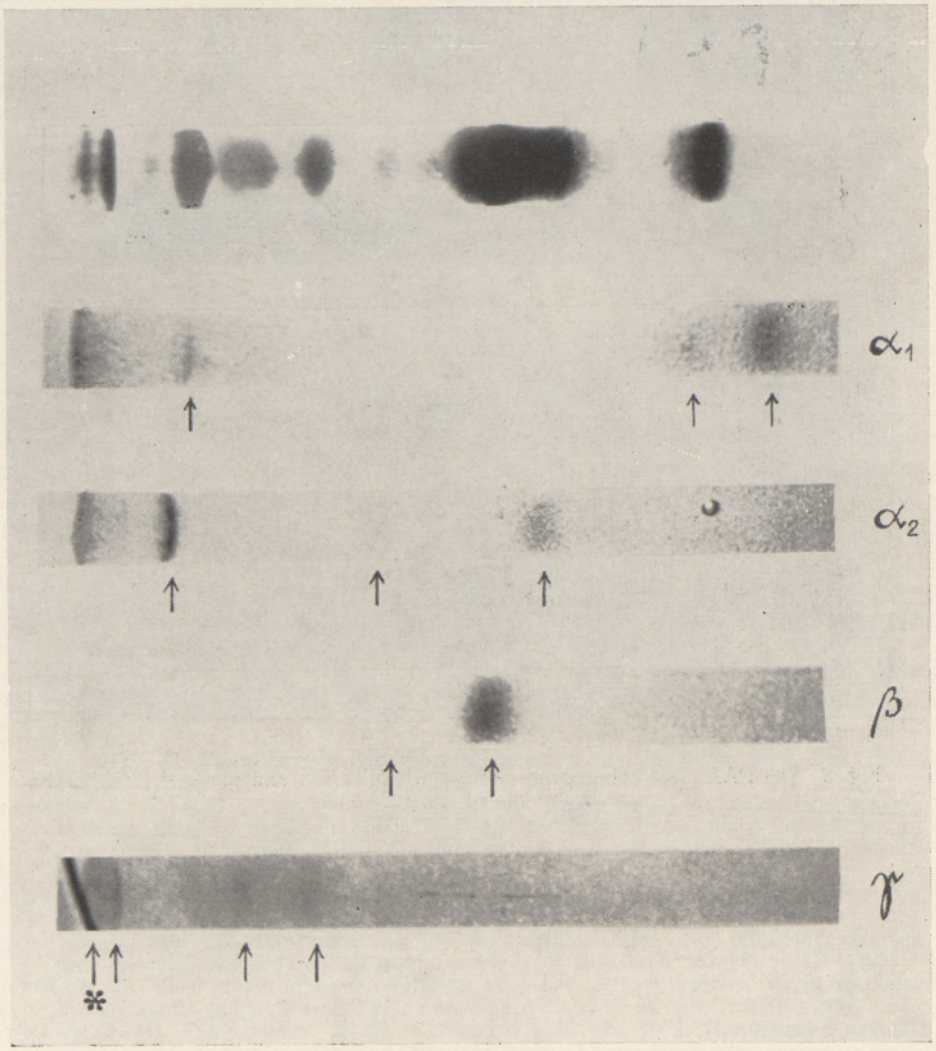

Fig. 1. The PAA electrophoregrams of the whole serum and paper-electrophoretic fractions of carp No. 4 (*— the edge of the Petri dish).

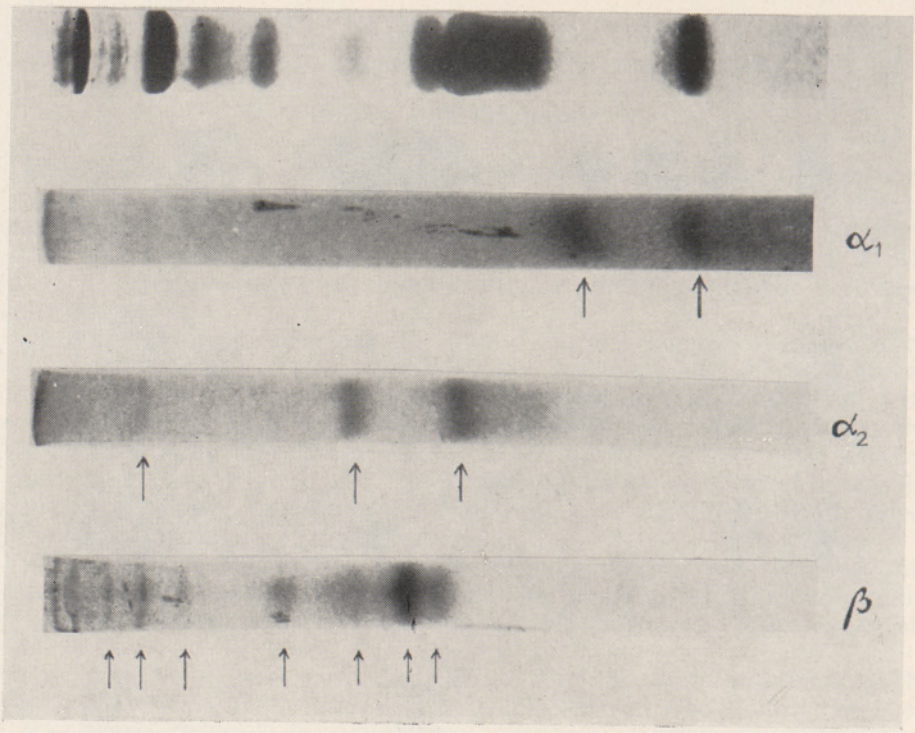

Fig. 2. The PAA electrophoregrams of the whole serum and paper-electrophoretic fractions of carp No. 5.

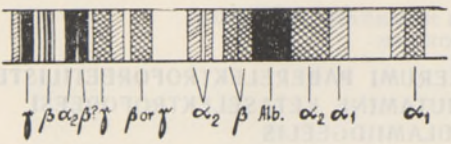

Fig. 3. The scheme of the localization of the main paper-electrophoretic fractions of carp serum by PAA gel electrophoresis.

Drilhon et al., 1962; Creyssel et al., 1964). It becomes evident that the distribution and disposition of the main fractions (prealbumins, albumin, transferrins, α_2F and α_2S globulins, β-globulins) is principally the same as in starch gel and in PAA gel. Consequently, the electrophoregrams obtained by these techniques should be comparable, in general.

Conclusions

1. The paper-electrophoretic fractions of the blood serum globulins of common carp are not homogeneous, and they divide by PAA gel electrophoresis into 2—5 fractions.
2. The fractions obtained by PAA gel electrophoresis are localized similarly to the fractions obtained by the starch gel electrophoresis, but the number of fractions detected was markedly greater. Some of very weak fractions are, evidently, not detectable by repeated electrophoresis.
3. The double fractionation of serum proteins can be taken as a basis at the innomination of the fractions obtained by each technique.

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KARPKALA (*CYPRINUS CARPIO*) VERESEERUMI PABERELEKTROFOREETILISTE VALGUFRAKTSIOONIDE EDASINE LAHUTAMINE KETASELEKTROFOREESI ABIL POLÜAKRÜÜLAMIIDGEELIS

Resümee

Paberelektroforeesi abil saadud karpkala vereseerumi valgufraktsioonide edasisel lahutamisel ketaselektroforeesil polüakrüülamiidgeelis saadi α_1-globuliinidest 2, α_2-globuliinidest 3—4, β-globuliinidest 3—5 ja γ-globuliinidest 2—3 fraktsiooni. Nende paigutus foregrammil üksteise suhtes on väga sarnane paigutusele tärklisgeel-elektroforegrammil.

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Аарe КИРСИПУУ

ДАЛЬНЕЙШЕЕ РАЗДЕЛЕНИЕ БУМАЖНО-ЭЛЕКТРОФОРЕТИЧЕСКИХ ФРАКЦИЙ БЕЛКОВ СЫВОРОТКИ КРОВИ КАРПА (*CYPRINUS CARPIO*) ПРИ ПОМОЩИ ДИСК-ЭЛЕКТРОФОРЕЗА В ПОЛИАКРИЛАМИДНОМ ГЕЛЕ

Резюме

Полученные при помощи электрофореза на бумаге фракции белков сыворотки крови карпа подвергали диск-электрофорезу в полиакриламидном геле. α_1-глобулины разделялись на 2, α_2-глобулины на 3—4, β-глобулины на 3—5 и γ-глобулины на 2—3 фракции. Расположение фракций на фореграмме сходно расположению их на фореграммах, полученных в крахмальном геле.

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