

Remigiusz WĘGRZYNOWICZ, Krystyna MARCZUK, Bernard KŁYSZĘJKO

Physiology

OSMOTIC FRAGILITY OF ERYTHROCYTES OF BREAM
ABRAMIS BRAMA (L.) FROM ZALEW SZCZECIŃSKI

STOPIEŃ WRAŻLIWOŚCI OSMOTYCZNEJ ERYTROCYTÓW
LESZCZA *ABRAMIS BRAMA* (L.) ZALEWU SZCZECIŃSKIEGO

Institute of Ichthyology*)

Studies on erythrocytes fragility of bream *Abramis brama* (L.) originating from Zalew Szczeciński proved that maximal hemolysis takes place in solution of 0,25% NaCl and minimal in 0,55%. The curves of erythrocytes osmotic resistance are of sigmoidal profile. Visual density in any concentrations lower then 0,25% NaCl corresponds to decreasing osmotic fragility proportionally to progress of dilutions.

The problem of erythrocytes resistance is connected with enzymatic mechanism, which in phenomenon of hemolysis, plays dominant role (Altman, 1959). Exhausting through hexozomonophosphate process of enzym reserves (glucoso-6 phosphate dehydrogenase) in erythrocytes causes some disturbances which limit the metabolic function; this is apparent in increased tendency to hemolysis. In phenomenon of physiological transformations, this is one of the chains observed particularly in ageing process of erythrocytes.

There is no doubt that the environmental factors influence the process of hemopoiesis. The environmental factors cause change of somatic system functional stress and of neurovegetative and hormonal regulation; this is apparent in stimulation of hemopoietic system in quantitative increase of new hemacytes and consequently in increase of erythrocytes osmotic resistance.

Studies on osmotic fragility of erythrocytes from homoiothermal and poikilothermic animals prove vital differences among the species (Feri, Perk, 1964; Smirnova, 1959; Urazbajew, 1968). From investigations performed on various species of sea fish from Mexican Bay, appears that the curves of erythrocytes osmotic fragility (per cent of hemolysis to degree of NaCl concentration) are of sigmoidal profile (like of people). Noted in these investigations were wide ranges of resistance in NaCl solution ranging from 0.34% to 0.70%. Increased fragility was in some cases noted within the same group of species with hemolysis taking place in such low NaCl concentration as 1% (George et al., 1969).

It is apparent from these investigations that the limits of fish osmotic fragility for the same groups of species, may vary very widely. There is no

*) From Ex-Department of Fish Physiology

doubt that such limits are determined, among other dominant factors, by the conditions of environment, as their influence on hemopoietic system had been proved by numerous investigation (Bernard, Ruffic, 1969).

Considerable varieties of environmental conditions in various regions of world, permit by comparative analysis, for causal hypothesis on phenomenon of variability in range of erythrocytes osmotic fragility.

Our initial studies show the differences in erythrocytes osmotic fragility appearing within the species of fish which remain under artificial environments of various concentration of NaCl. Both, excess or shortage of particular components may influence the hemopoietic system by introducing into organism the adaptive reactions influencing various chains of metabolism. It may be assumed that, next to other parameters, changes of erythrocytes resistance may be one of the indications on fish adaptive ability to changes of environment.

This study was framed by investigations on fish adaptability to environmental changes and was aimed to determine the degree of erythrocytes osmotic fragility of bream originating from "Zalew Szczeciński" in comparison with the results obtained on the same species of Aral Sea.

METHOD

The investigations were carried-out on grown-up specimens of bream males and females which had been fished in "Zalew Szczeciński" from July to September. Blood was taken with capillary from coccygeal artery into the test tubes previously washed with heparine. Until the beginning of investigations, but for period not exceeding two hours, the blood had been stored in ice. The measurement of erythrocytes fragility was performed according to method of Parpat and al. (1947) modified by George H. and al. (1968). Initially used for investigation of osmotic fragility was solution of 10% NaCl of pH 7.4 (in phosphate buffer). By diluting the initial solutions with distilled water obtained the working solutions of NaCl concentrations ranging from 0.00% to 1.0% in spacings of 0.05%.

The prepared solutions were incubated with blood during 40 minutes in temperature of 26°C and then after centrifuged at 220 rpm during 10 minutes. A sediment was removed before measurements. To determine the degree of hemolysis, optical density was examined with spectrophotometer (C. Zeiss, E-Germany) at wave length of 540 nm. Maximal hemolysis prevailed on solution of highest optical density.

RESULTS AND DISCUSSION

As appears from list of mean values (Tab.1), maximal hemolysis of bream erythrocytes prevails on solution density 336.9 at NaCl concentration of 0.25%. In solution of 0.30% NaCl, the erythrocytes hemolyse in 85%, while in solution of 0.45%, hemolysis occurs in 14.9% only. Minimal hemolysis of 6.0-3.9% is noted in NaCl concentrations between 0.50% and 0.55% (Tab.1).

Table 1

Osmotic fragility of erythrocytes of bream
Abramis brama (L.) in hypotonic solutions of NaCl

Concentration of NaCl in %	n	Optical density of Hb solution \bar{x}	d	\bar{x} in per cent of 336.9
0.00	39	276.9	+ 10.9	82.2
0.05	39	298.7	+ 13.2	88.6
0.10	39	320.1	+ 11.6	93.7
0.15	39	315.7	+ 10.9	95.0
0.20	39	334.3	+ 12.0	99.2
0.25	39	336.9	+ 13.4	100.0
0.30	39	286.4	+ 15.3	85.0
0.35	39	183.9	+ 19.4	54.6
0.40	37	107.2	+ 14.1	31.8
0.45	31	50.2	+ 8.7	14.9
0.50	11	20.3	+ 4.4	6.0
0.55	3	13.3	+ 3.6	3.9

d - standard deviation

\bar{x} - arithmetic mean

n - number of specimens examined.

The investigations performed at Aral Sea (Urazbaev, 1968) proved that the erythrocytes of bream hemolyse in 100.0% at 0.30% of NaCl concentration and do not hemolyse in solution of 40%. It is apparent from the above, that erythrocytes osmotic resistance of bream from "Zalew Szczeciński" comprises wider range when compared with the same species living in Aral Sea. This would indicate towards adaptive ability of this species to variable conditions of environment. Further comparative investigations relating to variations of environments and to other species of fish, shall permit for causal hypothesis on variability of phenomena in erythrocytes osmotic fragility range.

The curve of bream erythrocytes resistance (Fig. 1) (per cent of hemolysis related to degree of NaCl concentration) is of sigmoidal profile like in investigations of George and al. (1969) performed on sea fish of Mexican Bay. In distinction to the results presented by the above mentioned authors, our curve is supplemented by the values obtained in concentrations lower from maximal hemolysis. An analysis of the performed investigations indicates that optical density of erythrocytes in NaCl concentrations lower than 0.25% corresponds to slightly decreasing osmotic fragility towards progressive dilutions and attains in distilled water the values corresponding to 82.2% of hemolysis. According to investigation of George et al., hemolysis of 100% always appeared in certain concentrations of NaCl and remained at 100% in all lower concentration of salt. Optical density of erythrocytes in distilled water presented by the a/m authors, also did not correspond to 100% hemolysis.

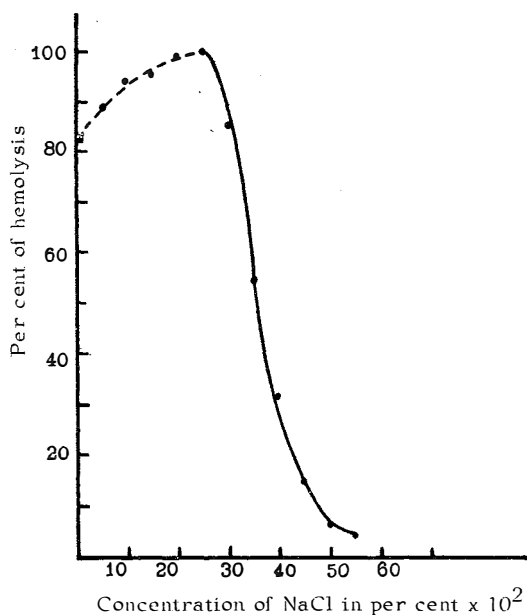


Fig.1. Erythrocyte hemolysis of bream *Abramis brama* (L.) in hypotonic solution of NaCl

Osmotic fragility gradually decreasing in lower concentration of NaCl shown by us requires further investigations. It is difficult to provide an uniform explanation if such phenomenon (also in distilled water) is due to not full hemolysis, or to precipitation of Hb macromolecular or partial absorption by the nucleic acids insoluble in water.

CONCLUSIONS

1. Maximal hemolysis of erythrocytes of bream from "Zalew Szczeciński" takes place in 0,25% NaCl and minimal in 0,55% solutions.
2. Osmotic resistance of erythrocytes of bream from "Zalew Szczeciński" shows wider range in comparison with the same species of Aral Sea.
3. Curve of osmotic resistance of bream (per cent of hemolysis to concentration of NaCl) is of sigmoidal profile.
4. Visual density of erythrocytes in NaCl concentration lower than 0,25% corresponds to decreasing osmotic fragility proportionally to progress of dilutions.

REFERENCES

- Altman I.K., 1959: Some enzymological aspects of the human erythrocyte., *Am. Journ. of Med.*, - 27, 936.

- Bernard I., Ruffic I., 1966: Hematologie géographique, Masson, Paris.
- Feri V.F., Perk K., 1964: Osmotic hemolysis of nucleated erythrocytes. - Expl. Cell. Res., 35: 65-69.
- George H., Ezell L., Sulya and Dodgen C.L., 1969: The osmotic fragility of some fish erythrocytes in hypotonic saline. Comp. Biochem. Physiol., 28: 409-415.
- Smirnova L.N., 1967: Ob osmotičeskoj resistennosti eritrocitov ryb. Wopr. Icht., 6: 1131-1134.
- Urazbajev S., 1968: Rezistennost eritrocitov ryb Aralskowo Morja k'solenosti. USSR Uz. Ilimler. Akad. Karakalp. Fil. Chabar., Viestn. Karakalp. Fil. AN UzSSR, 4/34:45-47.

Lityński T., 1966: Mikroelementy w życiu roślin, zwierząt i ludzi. [Microelements in life of plants, animals and human]. Kraków.

STOPIEŃ WRAŻLIWOŚCI OSMOTYCZNEJ ERYTROCYTÓW LESZCZA ABRAMIS BRAMA (L.) ZALEWU SZCZECIŃSKIEGO

Streszczenie

Badano oporność erytrocytów leszcza *Abramis brama* (L.) pochodzącego z Zalewu Szczecińskiego. W aspekcie oceny zdolności adaptacyjnych wyniki zestawiono z rezultatami uzyskanymi na tym samym gatunku żyjącym w Morzu Aralskim. Pomiar oporności przeprowadzono metodą Parparta i współprac. w modyfikacji Georga i współprac. przy zastosowaniu spektrofotometru.

W wyniku badania stwierdzono, że maksymalna hemoliza u leszcza z Zalewu Szczecińskiego występuje w 0,25%, minimalna w 0,55% roztworze NaCl, co stanowi szersze granice oporności ciałek czerwonych w zestawieniu z tym samym gatunkiem w Morzu Aralskim. Ponadto stwierdzono, że krzywe oporności osmotycznej krwinek leszcza (% hemolizy do stężenia NaCl) mają kształt sigmoidalny, a obraz gęstości optycznej ciałek czerwonych w stężeniach NaCl niższych od 0,25% odpowiada malejącej wrażliwości osmotycznej w miarę postępu rozcieńczeń.

СТЕПЕНЬ ОСМОТИЧЕСКОГО РЕЗИСТАНСА ЭРИТРОЦИТОВ ЛЕЩА АБРАМИС БРАМА (L.) ИЗ ЩЕЦИНСКОГО ЗАЛИВА

Резюме

Исследован резистанс эритроцитов леща *Abramis brama* (L.) обитающего в Щецинском заливе. С точки зрения оценки адаптационных способностей сопо-

ставлены результаты, полученные при исследовании леща из Щецинского залива, с результатами, полученными при исследовании этого же вида, обитающего в Аральском море. Определение резистанса проведено по методу Парпарта и его сотрудников, модифицированному Георгом и его сотрудниками, при использовании спектрофотометра.

В результате исследований установлено, что максимальный гемолиз у леща из Щецинского залива наблюдается в 0,25%, минимальный – в 0,55% растворе хлористого натрия, что составляет более широкие границы резистанса красных кровяных телец в сопоставлении с тем же видом, обитающим в Аральском море. Кроме того, установлено, что кривые осмотического резистанса кровяных телец леща (% отношения гемолиза к концентрации хлористого натрия) имеют сигмоидальную форму, а картина оптической плотности красных кровяных телец в концентрациях хлористого натрия, меньших, чем 0,25%, соответствует уменьшающемуся осмотическому резистансу по мере уменьшения концентраций.

Address:

Received 15.VII.1972

Doc. Dr Remigiusz Węgrzynowicz
Instytut Ichtiologii AR

Szczecin, ul. Kazimierza Królewicza 4
Polska - Poland