

CHAPTER - VIIINORGANIC IONS IN EMBRYONIC
STAGES OF CYPRINUS CARPIO

INTRODUCTION

Egg membrane being charged electronegative (Kaneda, 1936; Ikeda, 1937a), motility of cations is higher than that of anions. Gray (1913, '20) believed that retention of electrolytes in the egg was due to impermeability of protoplasmic membrane, which was considered polarized. Fertilization was said to destroy polarization and for a short period following sperm penetration the egg membrane was considered permeable to ions. Subsequently, membrane again becomes polarized and impermeable. Loeb & Hasteney (1915), Ikeda (1937b) and Yamamoto (1954)

held that metallic ions are closely linked to protein and other organic radicals, hence retained in the cell by chemical forces and not by an impermeable membrane; egg membrane is permeable to ions. Rudy and Potts (1959) found exchange of sodium ions between eggs of Salmon and its environment at different stages, exchange, however, was confined to caelion and perivitelline fluid in early stages of development. Concentrational differences created by active transport of Na^+ and K^+ provide functional osmotic and electrochemical gradient (Kernan, 1980). The interior of egg has moderately high percentage of electrolytes. Osmotic pressure of eggs being much higher than that of the surrounding media, Schwan and Gunnström (1912) observed that osmotic pressure of the egg falls after fertilization. Gray (1920), however, observed a marked evolution of electrolytes into surrounding medium, leading to precipitation of globulin following injury or death.

Sodium and potassium, besides their importance in providing functional osmotic and electrochemical gradient, serve as structure promoters by binding to anionic sites for both polynucleic acids and proteins. Both, specially potassium is considered important as an enzyme activator.

Calcium is said to be intimately involved in the activation process of fertilized eggs, and its

role may be related to its action on various enzymes (Yemamoto, 1954; Merrill, 1974). It is also said to be concerned with gelation of cytoplasm (Crachet, 1960). Magnesium and zinc ions serve as metallic cofactors for several important enzymes (Phipps, 1976).

The role of cations thus is proved in biological processes beyond any shade of doubt. The only work thus far on concentration levels of these cations during embryonic development relate to *Salmo gairdnerii* (Sogino & Yasuda, 1952). The present chapter deals with the levels of Na^+ , K^+ , Ca^{++} , Mg^{++} and Zn^{++} and assessment of their relative importance in the different stages of embryonic development of *Cyprinus carpio*.

METHODS:

MATERIALS USED:

Known weights of lyophilized samples of different embryonic stages were digested with concentrated nitric acid and proceeded for the analyses of inorganic ions by atomic absorption spectrophotometry.

RESULTS:

In atomic absorption spectrophotometry, a light beam is directed through the flame, into a

monochromator and onto a detector that measures the amount of light absorbed by the atomized element in the flame. The amount of light absorbed at a wavelength characteristic to a particular element is proportional to the concentration of element.

REAGENTS:

1. Stock solutions (Vogel, 1951)

Standard stock solutions of Na^+ , K^+ , Ca^{++} , Mg^{++} and Zn^{++} were prepared as follows:

1. Sodium solution: 2.5415 g of anhydrous NaCl dissolved in 1 litre of deionized distilled water; this gave 1000 μg of sodium/ml.
2. Potassium solution: 1.9090 g of KCl dissolved in 1 litre of deionized distilled water; this solution gave 1000 μg of potassium/ml.
3. Calcium solution: 2.4973 g calcium carbonate dissolved in dilute HCl and the whole solution made to 1 litre with deionized distilled water. This solution gave 1000 μg of calcium/ml.
4. Magnesium solution: 10.0135 g of magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) dissolved in 200 ml of deionized distilled water; added 1.5 ml concentrated nitric acid and made upto 1000 ml with deionized distilled water. One ml of this solution gave 1000 μg of magnesium.

5. Zinc solution: 1.000 g of zinc metal dissolved in 20 ml of diluted HCl ($\text{HCl}:\text{H}_2\text{O}$, 1:1 v/v) and made to 1,000 ml with deionized distilled water. One ml of this solution gave 1000 μg of zinc.

6. working standard solutions:

Working standard solutions of Na^+ , K^+ , Ca^{++} , Mg^{++} and Zn^{++} containing different concentrations (10, 25, 50, 75 and 100 $\mu\text{g}/\text{ml}$) were prepared by diluting appropriate volumes of stock solutions.

PROCEDURE:

Fresh eggs were washed thoroughly in deionized distilled water to remove adhering substances and lyophilized upto constant weight. Accurately weighed eggs of each sample were digested overnight in 2-4 ml of concentrated nitric acid. Digested samples were diluted appropriately with deionized distilled water. 5 ml from each aliquot was taken; either 1 ml of Li^+ (for Na^+ & K^+) or 1 ml of La^{++} solution (for Ca^{++} & Mg^{++}) was then added and the volume made to 10 ml with deionized distilled water. No releasing factor was added in case of Zn^{++} and the aliquot as such was taken for the analysis.

All these samples were aspirated and atomized in a flame and absorbances (or emission for Na^+)

measured using an atomic absorption spectrophotometer (Double beam, AA-575 series).

Ionic concentrations in each sample were expressed as mg/100 mg dry weight of eggs.

RESULTS:

Results obtained are shown in Table 24; fig. 45. Na^+ & K^+ concentrations were found to be much higher than Ca^{++} , Mg^{++} and Zn^{++} throughout the embryonic development.

K^+ remains nearly constant until gastrula stage; depletes very sharply during closing of blastopore stage; attains its initial value during comma and eye stages and then depletes slightly just before hatching. Na^+ concentration depletes sharply just after fertilization until blastodisc formation, gradually until gastrulation stage and again sharply during closing of blastopore. Like K^+ , its value increases at comma and eye stages and then depletes slightly just before hatching.

Ca^{++} remains nearly constant throughout the embryonic development. However, it shows some rise following fertilization and just prior to hatching. Mn^{++} follows nearly identical pattern of Ca^{++} . While Mg^{++} shows some depletion during closing of blastopore stage, it remains nearly constant in other stages.

Inorganic ions, Na^+ , K^+ , Ca^{++} , and Mg^{++} and in $log/100$ mg dry weight) in unfertilized eggs and in different developing stages, during embryonic development of Carinus carpio

S.No.	stages									
	Unfertilized eggs	Blasto-disc	Early morula	Late morula	Gastrula	Closing of blastopore	eyed	Prior to hatching		
1	0.640	0.332	0.309	0.267	0.296	0.278	0.091	0.276	0.259	0.241
2	0.672	0.331	0.341	0.275	0.300	0.297	0.092	0.275	0.258	0.238
3	0.640	0.359	0.297	0.227	0.185	0.253	0.086	0.271	0.238	0.158
Mean	0.650	0.377	0.315	0.256	0.260	0.259	0.079	0.274	0.251	0.212
± S.D.	±0.018	±0.016	±0.023	±0.026	±0.060	±0.033	±0.057	±0.002	±0.025	±0.047
1	0.515	0.900	0.801	0.756	0.821	0.725	0.073	0.792	0.742	0.698
2	0.781	0.743	0.816	0.698	0.715	0.733	0.159	0.774	0.761	0.621
3	0.802	0.711	0.764	0.677	0.722	0.671	0.157	0.733	0.768	0.644
Mean	0.799	0.811	0.793	0.711	0.752	0.709	0.129	0.766	0.757	0.654
± S.D.	±0.017	±0.080	±0.027	±0.042	±0.060	±0.034	±0.050	±0.030	±0.013	±0.040
1	0.060	0.105	0.105	0.105	0.089	0.114	0.066	0.093	0.082	0.128
2	0.057	0.103	0.094	0.100	0.105	0.084	0.118	0.098	0.098	0.148
3	0.058	0.126	0.092	0.100	0.118	0.107	0.115	0.130	0.109	0.137
Mean	0.058	0.111	0.097	0.101	0.104	0.101	0.099	0.103	0.096	0.137
± S.D.	±0.002	±0.013	±0.007	±0.002	±0.014	±0.015	±0.030	±0.024	±0.013	±0.010

Table - 24 (contd)

S.No.	tapes									
	Unfertili- lized eggs	lasto- disc	Early morula	Late morula	blastula	Gastrula	Closing of blastopore	eye	prior to hatching	
1	-	0.088	0.084	0.086	0.085	0.080	0.061	0.33	0.077	0.084
2	0.034	0.081	0.087	0.085	0.090	0.085	0.058	0.101	0.101	0.096
3	0.077	0.091	0.080	0.077	0.074	0.078	0.048	0.085	0.082	0.070
Mean	0.067	0.085	0.083	0.083	0.083	0.081	0.059	0.083	0.080	0.083
± 1 S.D.	±0.004	±0.002	±0.003	±0.005	±0.003	±0.003	±0.010	±0.003	±0.020	±0.013
1	-	0.027	0.036	0.035	0.035	0.037	0.043	0.037	0.038	0.058
2	0.013	0.020	0.033	0.037	0.047	0.039	0.044	0.058	0.052	0.053
3	0.018	0.024	0.058	0.037	0.040	0.036	0.042	0.059	0.045	0.064
Mean	0.015	0.023	0.042	0.036	0.040	0.037	0.043	0.038	0.045	0.058
± 1 S.D.	±0.003	±0.003	±0.014	±0.001	±0.006	±0.001	±0.001	±0.001	±0.007	±0.005

DISCUSSION

Gray (1920) observed exosmosis of electrolytes in trout eggs following injury or death. He considered the capacity of injured or dead eggs to be due to precipitation of globulin, held in solution by acid or alkali, whereas swelling is due to increased osmotic pressure. The present author has observed identical phenomenon in Cyprinus carpio eggs. Relatively high concentrations of Na^+ and K^+ in the unfertilized eggs of C. carpio thus becomes intelligible. Aside this, these cations are important in active transport and maintenance of protein and nucleic acid conformations. Further, K^+ is a well known enzyme activator and is also implicated in cellular replication and protein and purine biosynthesis (Kernan, 1980).

Gelation of cytoplasm, uptake of phosphates in consequence of increased activities of ATPase and protease and oxygen consumption markedly increase following fertilization (Brachet, 1960). Increase in Ca^{++} concentration at fertilization in C. carpio is clearly related to activation process, gelation of cytoplasm (Brachet, 1960) and activation of ATPase, besides contributing to low passive permeability (Whipps, 1975) of the egg. Na^+ efflux, steeply at fertilization and gradually thereafter until late morula stage (Fig. 45), is on account of gelation of cytoplasm. Accumulation of Na^+ would,

on the other hand, cause solation and increased water permeability (Fujii, 1969).

The most interesting observation, however is the very low concentration of both Na^+ & K^+ at closing of blastopore stage. Glycogenolysis & conversion of yolk into cytoplasmic proteins or ribonucleoproteins are known to occur following gastrulation. Free ionic levels of Na^+ and K^+ are bound to rise resulting in toxicity to embryo (Ikeda, 1937a). Large scale efflux of Na^+ and K^+ by active transport is visualized.

During comma & eye stages, a wide array of new proteins and enzymes are synthesized to promote differentiation and morphogenesis (Brachet, 1960). active uptake of Na^+ and K^+ during these stages is thoroughly convincing. Na^+ accumulation is additionally important at these stages for osmoregulation (Whipps, 1976).

Developmental activity having reduced considerably just before hatching, a slight fall in Na^+ & K^+ levels is natural. Ca^{++} however, would be required for imparting low passive permeability at this stage, hence the slight observed increase in its level. The levels of Zn^{++} and Mg^{++} , which generally act as coenzymes, however, are found relatively invariable.