ON REGULATION OF CONCENTRATION AND CONTENT OF CHLORIDE IN GOLDFISH

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Only little is known about the variations which may normally occur in the content of water and ions of fresh water fishes. Such variations are determined by the rates at which water and ions are taken up or lost. Water is continuously being absorbed through the outer surfaces, various ions may be taken up actively by the gills or ingested with the food. Loss takes place through the kidneys and the surface. The renal loss varies with the urine flow and ionic concentration of the urine which is generally very dilute. Loss of ions by diffusion through the skin also varies. It is for instance strongly increased in disturbed or wounded fish (Krogh, 1937; Meyer, 1948).

The capacity for uptake of sodium and chloride ions from dilute solutions is highly developed in many fresh water fishes (Krogh, 1937, 1939; Meyer, 1948, 1951; Mullins, 1950; and Wikgren, 1953). In the stickleback *Gasterosteus aculeatus* Mullins showed that one quarter of the amount of sodium in the body is exchanged per day. The osmotic and ionic concentration is, however, kept rather constant despite a high rate of turn-over of ions. The osmotic pressure is about 300 m.osM./l in fresh water teleosts (Duval, 1925; Krogh, 1939; Vinogradov, 1953; Robertson, 1954). Variations in content of sodium and chloride ions—if they occur to any degree—are therefore presumably accompanied by such variations in water content that the osmotic pressure of the body fluid remains constant.

In the present investigation we followed during several weeks the daily changes in chloride content and weight of undisturbed starving goldfish. From these data it was possible to estimate daily and long-term changes in osmotic pressure and in total content of water and chloride of the body. The chloride content of the body was also measured by the dilution method using the radioactive isotope Cl^{36}. Changes in chloride content could thus be compared with the total amount of exchangeable chloride in the fish.

Technique

Healthy, 10–20 g., goldfish were used. They were kept in aerated chambers of the type previously described (Jørgensen, Levi and Ussing, 1946). The experiments were performed during the months of March to May in an unheated room where the temperature varied from 15 to 20° C.

Daily variations in chloride content of the fish were calculated from changes in chloride content of the medium, which was 200 ml. tap water, of initial concentration about 1–2 m.eq./l. Chloride was determined in one-ml. samples according to Rehberg in Schnohr's (1934) modification. The chloride content of the medium was estimated with an accuracy of about 5 μeq. Only changes amounting to some 10 μeq. and more in chloride content are therefore significant.

Great care was taken not to disturb the fish unduly during the weighings, because handling itself can induce loss of weight and of chloride. The fish, which apparently got accustomed to the weighing procedure, were carefully transferred from their chambers into nets which were drained on filter paper. From the nets the fish were put into weighing-glasses without being touched directly by the hands. Weighings were made in duplicate. The fish were returned to water between the two weighings which were thus performed under the same conditions. The weight in grams was read with two decimals and rounded to 0 or 5. The duplicates deviated maximally by 100 mg. Daily changes in weight should therefore amount to 0.2 g. or more in order to be significant.

Total exchangeable chloride was determined in three well-nourished goldfish which were kept in 200 ml. tap water enriched with Cl^{36}-labelled sodium chloride. Changes in specific activity of the chloride in the medium were followed. From the

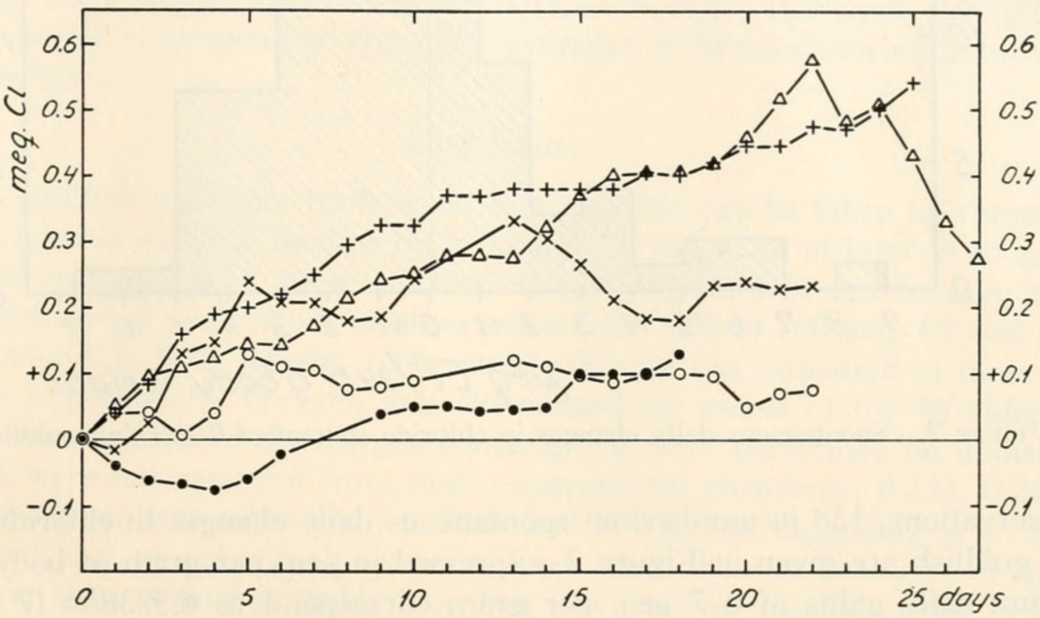

FIGURE 1. Spontaneous changes in chloride content of 5 starving goldfish, initially weighing 11–19 g.

degree of dilution of Cl^{36} the amount of chloride in medium plus goldfish was computed. Radioactivity was measured on evaporated 0.5-ml. samples of the medium by means of a Geiger-Müller end-window counter.

RESULTS

Variations in chloride content were followed during several weeks in five goldfish. As may be seen from Figure 1, uptake or loss of chloride often continued for several days or even weeks at a time, resulting in large changes in total chloride content of the body. One fish, weighing 19 g., took up 600 μeq. of chloride in 27 days, and another one, weighing 17 g., took up 575 μeq. in 22 days. Total exchangeable chloride, determined in three fed goldfish, was found to be 34.5, 35.5 and 43.0 μeq./g. body weight. Assuming an initial chloride content of 38 μeq./g., the two above mentioned fish thus increased their chloride content by 83 and 89 per cent, respectively.

Even the day-to-day changes in chloride content can be considerable. All avail-

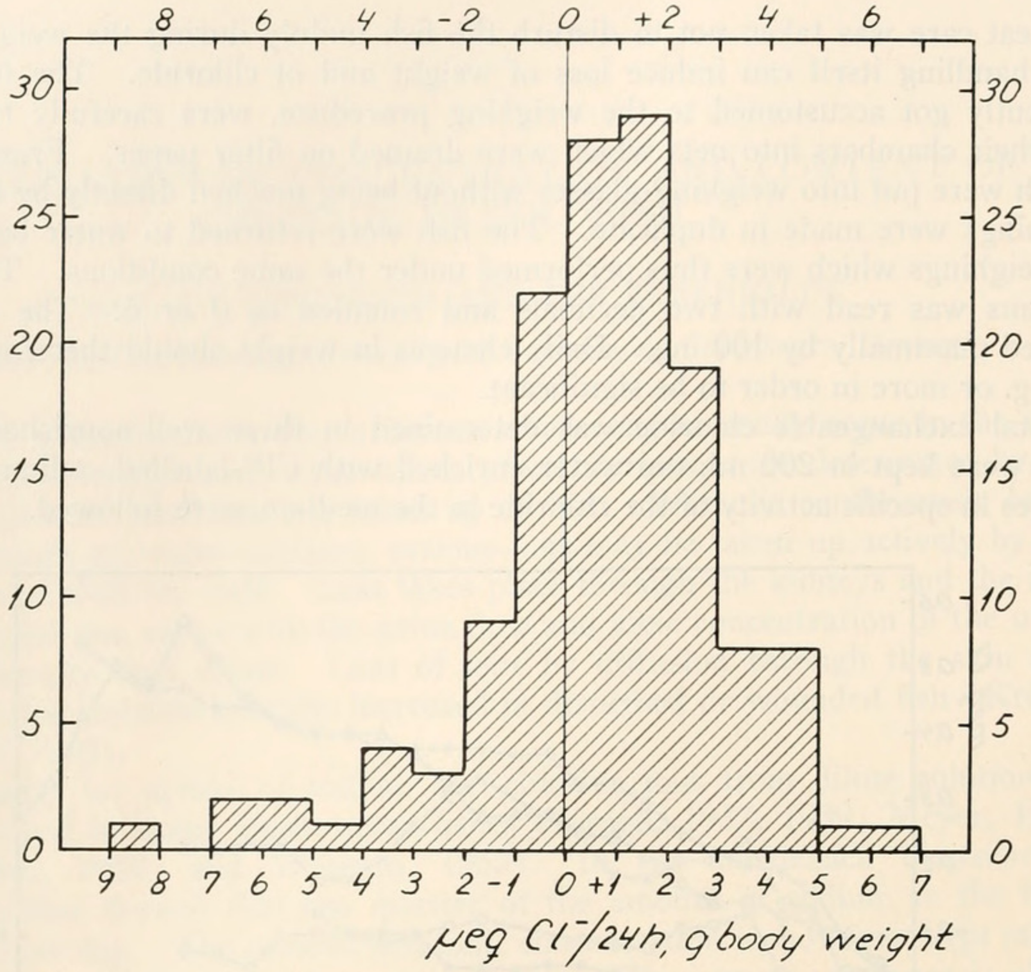

FIGURE 2. Spontaneous daily changes in chloride content of 9 starving goldfish.

able observations, 138 in number, on spontaneous daily changes in chloride content of nine goldfish are given in Figure 2, expressed in μeq. per gram of body weight. Maximum daily gains of 6–7 μeq. per gram correspond to $6.5/38 = 17$ per cent change in chloride content and maximum loss of 8–9 μeq. to some 22 per cent. Without proportional change in water content such large variations in chloride content would result in a strongly varying osmotic pressure. The daily changes in weight were therefore measured simultaneously with the daily changes in chloride content in order to estimate how constantly the osmotic pressure was maintained. The animals were adapted to the experimental conditions for ten days prior to the

TABLE I

Regression of daily weight changes (g.) of starving goldfish on daily changes in chloride content (m.eq.)

Experiment No.	Regression equations	Correlation coefficients	Statistical significances	Maximal daily changes in Cl content (μeq.)
1	$y=6.3x-104$	0.719	$0.02>P>0.01$	$-45+23$
2	$y=6.7x-112$	0.762	$0.01>P>0.001$	$-53+54$
3	$y=4.2x-97$	0.645	$0.05>P>0.02$	$-46+41$
4	$y=1.4x-108$	0.320	$0.3>P>0.2$	$-9+46$
5	$y=5.1x-216$	0.867	$P<0.001$	$-87+64$

measurements which were extended over a period of 11–15 days. A statistically significant correlation was found between daily chloride and weight changes in four of the five goldfish under experiment (Table I). In experiment 4 without significant correlation the daily variations were small. It may be seen from the table that the larger the daily changes the more significant was the correlation.

The regression coefficients in experiments 1, 2, 3 and 5 are 4.2, 5.1, 6.3 and 6.7. The mean value is 5.6. Thus, a change in chloride content of one m.eq. was accompanied by a change in weight of 4.2 to 6.7 g. Or in other words retention or excretion of 150 to 238 μeq. Cl was followed by retention or excretion of one ml. water.

The regression equations further show that in the five goldfish a chloride-independent loss of weight took place, ranging from 97 to 216 mg. daily. Computed per 100 g. body weight the values are 0.64, 0.75, 0.87, 0.88 and 1.34 g. which probably mainly correspond to the daily averages of break-down of tissue due to the metabolism.

Discussion

In the goldfish and other fresh water fish, chloride can be taken up through the gills and ingested with the food or by drinking. It might be of interest to estimate the relative importance of these sources. From Figure 2 it can be seen that in some 25 per cent of the periods of observation from 200 to 700 μeq. Cl was gained per day and 100 g. body weight. The metabolic rate was measured in three of the fish under experiment. Oxygen was determined by means of the Winkler technique. Measurements of the oxygen consumption were performed on undisturbed fish which were not removed from their experimental chambers; 0.233, 0.240 and 0.237 l. of oxygen was consumed per 24 h. and 100 g., corresponding to a combustion of about ¼ g. mixed organic matter. Even when the metabolic rate of starving fish is lower than normal probably less than one gram of organic matter is needed daily in order to cover the energy requirements. If one gram were to be obtained in the form of animal food containing, say, 70 per cent water and 40 μeq. Cl/g., this would correspond to an ingested amount of 130 μeq. Cl per 24 h. and 100 g., or only a quarter of an amount which is easily taken up through the gills. Presumably much less than 40 μeq. Cl/g. is contained in the food of the goldfish which eat much plant material, partly decaying, and poor in chloride.

Recent investigations of Allee and Frank (1948, 1950) show that some drinking takes place in fresh water fishes. Chloride uptake by drinking can, however, only be negligible in the goldfish. The daily diuresis amounts to some 10 ml. per 100 g. Most of this water is probably taken up through the outer surface, especially the gills (Krogh, 1939). Thus, at most a few μeq. Cl can be obtained by drinking in ordinary fresh water which generally contains less than one m.eq. Cl per liter. Under normal conditions, therefore, the gills must be more important organs for absorption of chloride than is the alimentary tract.

The total amount of chloride in the body is not precisely regulated in the goldfish, at least not in starving animals, whose chloride content could be almost doubled. The chloride concentration and osmotic pressure are better regulated. A loss or gain of only some 0.3 m.eq. Cl/100 g. within 24 hours generally coincides with a corresponding loss or gain of water (Table I). An increase in chloride content of 0.3 m.eq. per 100 g. body weight would produce an increase in osmotic pressure of about 3 per cent if no compensatory changes took place, assuming body water to be 70 per cent of the body weight and osmotic pressure to be equivalent to 300 m.osM./l. The osmoregulatory mechanisms are therefore probably sensitive to variations smaller than ±3 per cent of the osmotic pressure when such change occurs in 24 hours or less.

It was found that loss or gain of 150 to 238 μeq. Cl corresponded to a change in body water of one ml. However, the chloride concentration of the body fluid is lower than 150 m.eq./l, probably about 117 m.eq. as found by Robertson (1954) in the powan *Coregonus clupeoides*. Periods of steady uptake of chloride must consequently result in increasing concentrations of chloride and presumably also osmotic pressure of the body fluid. Periods of loss, on the other hand, must ultimately lead to decreasing chloride and osmotic concentrations. It thus seems as if the levels at which chloride and osmotic concentrations are maintained vary with the total chloride content of the body. It can further be assumed that the increase in chloride or osmotic concentrations which follows chloride uptake stimulates mechanisms that eventually result in net loss of chloride. Similarly, decreases in chloride or osmotic concentrations may be supposed finally to stimulate uptake and to reduce excretion of chloride, so that body chloride is once more increasing or perhaps kept constant for some time. Volume and osmotic regulation thus seem to be mutually dependent within a system that permits much larger variations in chloride content than in chloride concentration and consequently also osmotic pressure.

Summary

Daily changes in chloride content and weight of undisturbed starving goldfish were followed during several weeks. Uptake or loss of chloride often continued for several days or even weeks at the time. Up to 83 and 89 per cent increases in chloride content were measured. Maximum daily gains were 17 per cent, and losses 22 per cent of total chloride content. A statistically significant correlation was found between daily chloride and weight changes. Retention or excretion of 150 to 238 μeq. Cl was followed by retention or excretion of one ml. water.

LITERATURE CITED

Allee, W. C., and P. Frank, 1948. Ingestion of colloidal material and water by goldfish. *Physiol. Zoöl.*, **21**: 381–389.

Duval, M., 1925. Recherches physico-chimiques et physiologiques sur le milieu interieur des animaux aquatiques. *Ann. Inst. Océanogr.*, **2**: 233–407.

Frank, P., and W. C. Allee, 1950. Ingestion of colloidal thorium dioxide by representative minnows from the Chicago region. *Physiol. Zoöl.*, **23**: 134–139.

Jørgensen, C. B., H. Levi and H. H. Ussing, 1946. On the influence of the neurohypophysial principles on the sodium metabolism in the axolotl *Amblystoma mexicanum*. *Acta Physiol. Scand.*, **12**: 350–371.

Krogh, A., 1937. Osmotic regulation in fresh water fishes by active absorption of chloride ions. *Zeitschr. vergl. Physiol.*, **24**: 656–666.

Krogh, A., 1939. Osmotic regulation in aquatic animals. Cambridge.

Meyer, D. K., 1948. Physiological adjustments in chloride balance of the goldfish. *Science*, **108**: 305–307.

Meyer, D. K., 1951. Sodium flux through the gills of goldfish. *Amer. J. Physiol.*, **165**: 580–587.

Mullins, L. J., 1950. Osmotic regulation in fish as studied with radio isotopes. *Acta Physiol. Scand.*, **21**: 303–314.

Robertson, J. D., 1954. The chemical composition of the blood of some aquatic chordates, including members of the Tunicata, Cyclostomata and Osteichtyes. *J. Exp. Biol.*, **31**: 424–442.

Schnohr, E. A., 1934. Study in the cause of death in high intestinal obstruction. Diss., Copenhagen.

Vinogradov, A. P., 1953. The elementary chemical composition of marine organisms. New Haven.

Wikgren, Bo-J., 1953. Osmotic regulations in some aquatic animals with special reference to the influence of temperature. *Acta Zool. Fennica*, **71**: 1–102.