

## THE EFFECT OF OSMOTIC AND ION-OSMOTIC STRESSES ON THE BLOOD AND URINE COMPOSITION AND URINE FLOW OF RAINBOW TROUT (*SALMO GAIARDNERI*)

PAUL T. KOSTECKI\*

School of Natural Resource, University of Michigan, Ann Arbor, MI 48109, USA

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**Abstract**—1. Changes in urine and plasma concentrations (sodium, potassium, magnesium, calcium and total osmotic) and urine production were determined in fish exposed to various concentrations of an ionically active substance, sodium chloride, and a non-electrolyte, mannitol, as well as freshwater.

2. Responses occurred for the most part over a short crisis period preceding establishment of new stable conditions.

3. It was shown that plasma homeostasis was not maintained in response to changing ion-osmotic and osmotic gradients.

4. Urinary osmotic and ionic concentrations were unaffected and urine production was shown to be inversely related to the external concentration.

5. It is suggested that ionic shifts between body compartments are an important aspect of ion-osmotic adaptation.

### INTRODUCTION

Several studies have been made on ionic and osmotic regulation in fish (Gordon, 1963; Weisbart, 1967; Wood and Randall, 1973a, b; Beyenbach and Kirschner, 1975; Talbot *et al.*, 1982; Holeton *et al.*, 1983; King and Goldstein, 1983). Most of this work has been concerned with regulation in fish fully adjusted to freshwater or seawater and then exposed to a salinity challenge. Few observations have been made on regulatory changes during the transition period for fish rapidly transferred to different ion-osmotic concentrations (Bath and Eddy, 1979; Kostecki, 1979, 1982).

The purpose of this paper is to determine the ionic and osmotic responses during ion-osmotic regulation in fish exposed to various ion-osmotic (sodium chloride) and osmotic (mannitol) concentrations during the initial exposure and after 7 days of acclimation.

### MATERIALS AND METHODS

Hatchery rainbow trout (*Salmo gairdneri*), weighing 100–250 g, were held in dechlorinated freshwater for at least 4 weeks prior to experimentation. Fish were fed daily, to satiation, with Purina trout chow and acclimated and tested at a water temperature of  $15.0 \pm 0.1^\circ\text{C}$ . The fish were starved for 7 days prior to any given experiment.

Experiments were performed to measure the responses of plasma and urine osmolality and ionic composition to changes in external osmotic (mannitol) and ion-osmotic (sodium chloride) concentrations at ambient concentrations above (250 and 500 milliosmoles (mOsm)) and below (deionized and freshwater and 10 mOsm) the plasma isosmotic level. Observations on blood covered a short-term (36 hr) and long-term (7 days) period, and on urine, a 4 day period.

Two types of experiments were performed to minimize any stress effects due to sampling. One group of fish was used to measure ion-osmoregulation changes in the blood (blood group) under various ion-osmotic concentrations,

while a second group was used to measure changes in the urine (urine group). The blood group was further divided into a short-term blood group and a long-term blood group. The long-term blood group and urine group were used to measure changes over 7 and 4 days of exposure, respectively. The short-term blood groups allowed for more precise sampling during the first 36 hr when most blood changes probably occurred in fish exposed to sodium chloride (Kostecki, 1978).

The experimental solutions, sodium chloride or mannitol, were made by adding the appropriate chemical to deionized water to yield the necessary concentrations. Samples of the deionized water as well as the experimental solutions were analyzed to insure proper osmotic and ionic (sodium, potassium, calcium and magnesium) concentrations. In the experimental solution, the calcium concentration was  $1.5 \text{ mEq l}^{-1}$  and the potassium and magnesium concentrations were essentially zero. The sodium concentration was zero in the mannitol media, but, of course, varied in the sodium chloride solutions. The calcium, magnesium and potassium gradients between the fish and the external environment remained constant despite the changes in osmolality.

### Blood

Fish, weighing 100–150 g, for the long-term experiment were transferred to 22, 16-l polypropylene tanks at a density of seven fish/tank. The fish were allowed to acclimate to the test tanks in aerated freshwater for 24 hr. The experiments were started after this acclimation period with an initial sample (Day 0) just prior to changing the external concentration. The tanks were then divided into seven groups of: distilled water and three concentrations each of sodium chloride and mannitol of 10, 250 and 500 mOsm.

Usually, five fish, with two exceptions, were sampled from each concentration after 1, 2, 4, and 7 days of exposure. Mortality dictated that only four fish were sampled from the 250 mOsm sodium chloride group on the seventh day, and three and two fish from the 500 mOsm sodium chloride group on days 4 and 7, respectively.

Fish for the short-term experiment were transferred to 20 similar tanks at a density of five to six fish/tank. They were allowed to acclimate to the tanks for 24 hr in freshwater. After the acclimation period an initial sample was taken and the tanks divided into two groups; freshwater and

\*Present Address: Zoology Department, University of Massachusetts, Amherst, MA 01003, U.S.A.

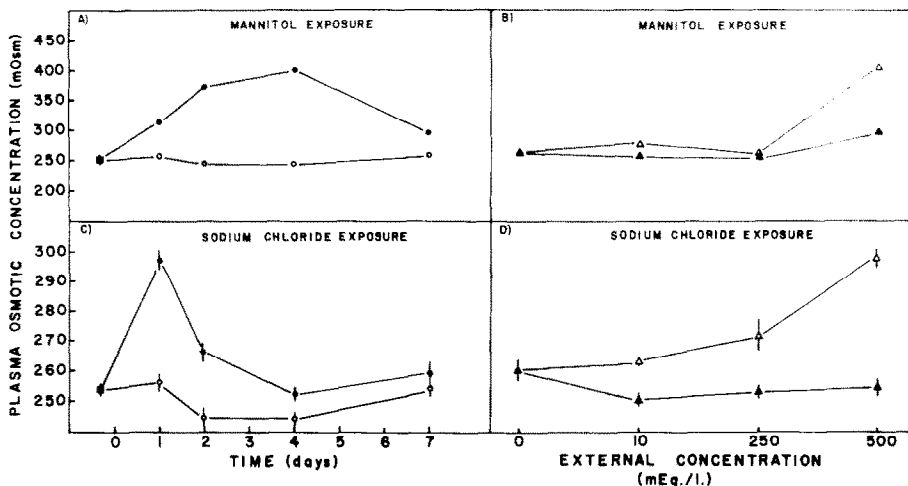


Fig. 1. Typical responses of rainbow trout exposed to mannitol and sodium chloride. (A) Exemplary relationship between plasma osmotic concentration and time for fish exposed to ambient concentrations of 500 mOsm mannitol (shaded symbols) and deionized water (unshaded symbols). (B) Relationship between maximum plasma osmotic concentration (unshaded symbols) during exposure of fish to various ambient mannitol concentrations and levels after 7 days (shaded symbols). (C) Exemplary relationship between plasma osmotic concentrations and time for fish exposed to ambient concentrations of 500 mOsm sodium chloride (shaded symbols) and deionized water (unshaded symbols). (D) Relationship between maximum plasma osmotic concentration (unshaded symbols) during exposure of fish to various ambient sodium chloride concentrations and level after 7 days (shaded symbols). Bars  $\pm$  2 S.E.,  $n = 5$  except (C)  $n = 3$  for Day 4 and  $n = 2$  for Day 7 for 500 mOsm sodium chloride, (D)  $n = 4$  for 250 mOsm and  $n = 2$  for 500 mOsm.

200 mOsm sodium chloride. Five fish were sampled from each concentration every 4 hr for 36 hr.

The medium in each tank for both sets of experiments was changed every 24 hr to prevent the buildup of waste products. Blood samples were obtained by cardiac puncture from anesthetized (MS 222; 1:20,000) fish. Ammonium heparin was used to prevent clotting and hemolysis. The blood samples were centrifuged at 5000 rev/min for 10 min  $\pm$  1 min. The plasma portion was stored in acid-washed, polypropylene vials at  $-20^{\circ}\text{C}$  until analysis. Hematocrit was also measured.

#### Urine

Urinary catheters were implanted in fish, 150–250 g, using the technique described by Kostecki (1978). The technique was improved by the construction of catheters from Silastic medical grade tubing (0.030  $\times$  0.650 in). After implanting the catheters the fish were transferred to respirometers (Kostecki, 1979) allowing urine collection in 50 ml volumetric flasks. Urine was collected for fish exposed to: freshwater, deionized water and three concentrations each of sodium chloride and mannitol of 10, 250 and 500 mOsm. Urine samples were collected from five fish from freshwater and four fish from each of the other concentrations except for three fish from the 500 mOsm sodium chloride group because of mortality. The fish were exposed to freshwater for 4 days prior to exposure to the test concentrations, enabling recovery from the implantation (Kostecki, 1978). The 24-hr cumulative urine flow rate was measured daily for 4 days. The urine samples were stored in acid-washed polypropylene bottles at  $-20^{\circ}\text{C}$  until analysis for urine osmotic concentration and ionic composition.

#### Analytical procedures

Osmotic concentrations of urine and plasma samples were determined against appropriate dilutions of prepared standards using a Model 512A, Wescor vapor pressure osmometer. Ionic analysis of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$  determinations, both standards and unknowns were diluted with lanthanum solution (0.1% w/v La) to avoid  $\text{Ca}^{2+}$  interference. The significance of the differences among means of

samples was tested using a multiple range test, Student–Newman–Kuel, at the  $P < 0.05$  level.

#### RESULTS

Plasma osmotic concentration of fish exposed to sodium chloride increased proportionally to the external concentration over the first 1–2 days, as illustrated for the 500 mOsm sodium chloride group in Fig. 1(C, D) but returned to pre-exposure levels after 7 days. These plasma osmotic increases were primarily due to increases in sodium and potassium concentrations [Figs 2(C), 3, 4]. Plasma calcium and magnesium concentrations decreased in fish exposed to the hypo- and hyper-ion-osmotic sodium chloride concentrations (Fig. 4).

Plasma osmotic concentrations were unaffected by external hypo-osmotic (0 and 10 mOsm) and slightly hyper-osmotic (250 mOsm) concentrations of mannitol and increased significantly ( $P < 0.05$ ) at the hyper-osmotic concentration (500 mOsm) [Figs. 1(A, B)] but without a parallel increase in any of the plasma ions measured [Figs 2(A, B)]. Plasma sodium, potassium and magnesium in fish exposed to various mannitol concentrations remained fairly constant, but plasma calcium decreased significantly ( $P < 0.05$ ) in response to the various mannitol concentrations [Figs 2(B), 5 and 6].

The initial changes in plasma osmotic and ionic concentrations and hematocrit were evaluated in greater detail over the short-term (0–36 hr) for fish exposed to an elevated sodium chloride concentration (200 mOsm). Plasma osmotic concentration of fish exposed to sodium chloride increased to levels significantly higher ( $P < 0.05$ ) than fish exposed to freshwater after 4 hr [Fig. 7(A)]. Thereafter, plasma osmotic concentration decreased to levels only

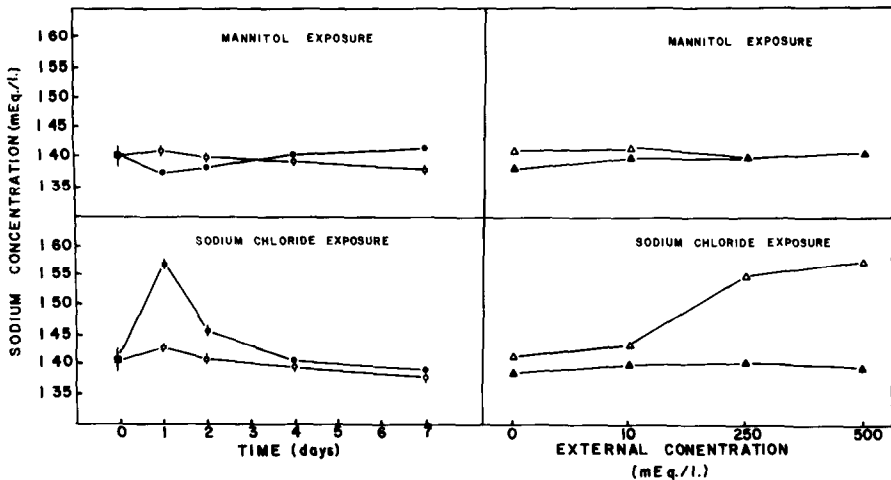


Fig. 2. Typical responses of rainbow trout exposed to mannitol and sodium chloride. (A) Exemplary relationship between plasma sodium concentration and time for fish exposed to ambient concentrations of 500 mOsm mannitol (shaded symbols) and deionized water (unshaded symbols). (B) Relationship between maximum plasma sodium concentration (unshaded symbols) during exposure of fish to various ambient mannitol concentrations and levels after 7 days (shaded symbols). (C) Exemplary relationship between plasma sodium concentration and time for fish exposed to ambient concentrations of 500 mOsm sodium chloride (shaded symbols) and deionized water (unshaded symbols). (D) Relationship between maximum plasma sodium concentration (unshaded symbols) during exposure of fish to various ambient sodium chloride concentrations and levels after 7 days (shaded symbols). Bars  $\pm$  S.E.,  $n = 5$  except (C)  $n = 3$  for Day 4 and  $n = 2$  for Day 7 for 500 mOsm sodium chloride, (D)  $n = 4$  for 250 mOsm and  $n = 2$  for 500 mOsm.

slightly higher than the control and pre-exposure levels by 36 hr. The higher plasma osmotic concentration of fish exposed to the sodium chloride appears to be due, in part, to an increase in plasma sodium [Fig. 7(B)] and, to a lesser extent, plasma potassium [Fig. 7(C)]. Plasma calcium [Fig. 7(D)] and magnesium [Fig. 7(E)] concentrations in fish exposed to

sodium chloride decreased and remained significantly lower ( $P < 0.05$ ) than the controls after 12–16 hr of exposure. No difference in calcium and magnesium gradients existed between the freshwater and the sodium chloride medium that could explain this change. Since absolute concentration changes were small, these ions may be qualitatively involved in

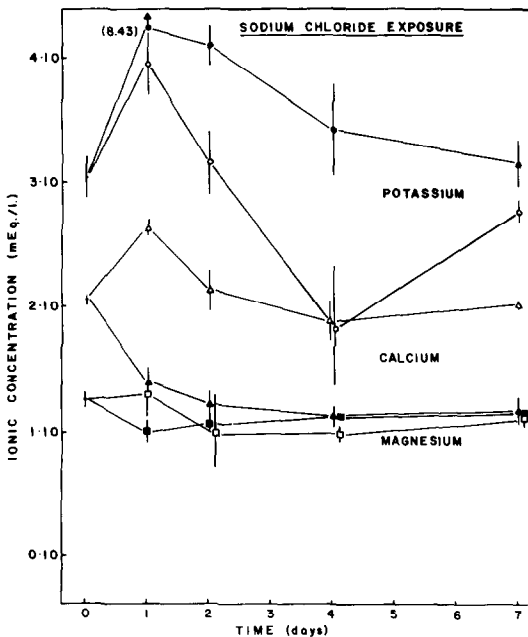


Fig. 3. Exemplary relationship between plasma potassium, calcium and magnesium concentrations and time for rainbow trout exposed to ambient concentrations of 500 mOsm sodium chloride (shaded symbols) and deionized water (unshaded symbols). Bars  $\pm$  2 S.E.,  $n = 5$  except  $n = 3$  for Day 4 and  $n = 2$  for Day 7 for 500 mOsm sodium chloride.

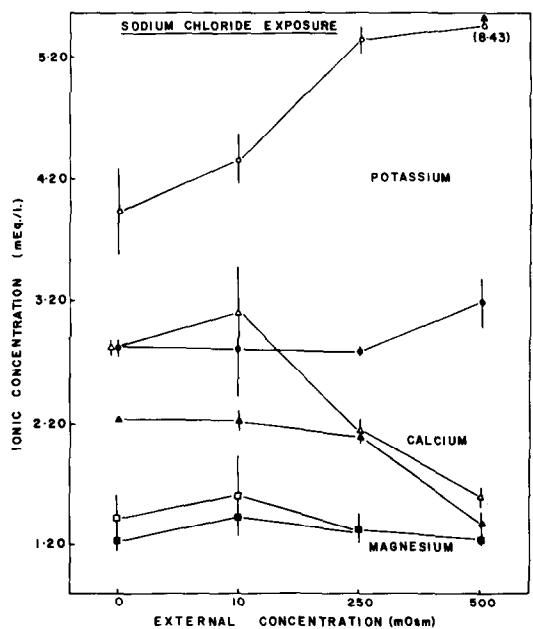


Fig. 4. Relationship between maximum plasma potassium, calcium and magnesium concentrations (unshaded symbols) during exposure of rainbow trout to various sodium chloride concentrations and levels after 7 days (shaded symbols). Bars  $\pm$  2 S.E.,  $n = 5$  except  $n = 4$  for 250 mOsm and  $n = 2$  for 500 mOsm.

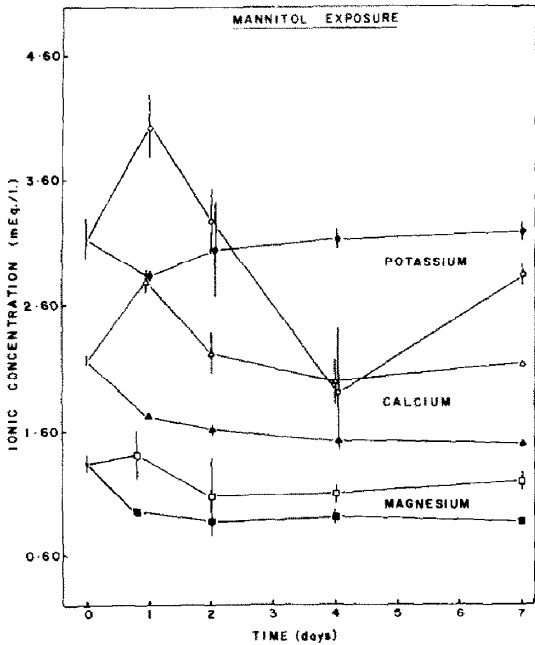


Fig. 5. Exemplary relationship between plasma potassium, calcium and magnesium concentrations and time for rainbow trout exposed to ambient concentrations of 500 mOsm mannitol (shaded symbols) and to deionized water (unshaded symbols). Bars  $\pm$  2 S.E.,  $n = 5$ .

osmotic or ion-osmotic regulation, perhaps by affecting membrane permeability.

Fish exposed to sodium chloride had significantly lower ( $P < 0.05$ ) hematocrits after 8 hr of exposure [Fig. 7(F)] than the control fish for the duration of the experiment.

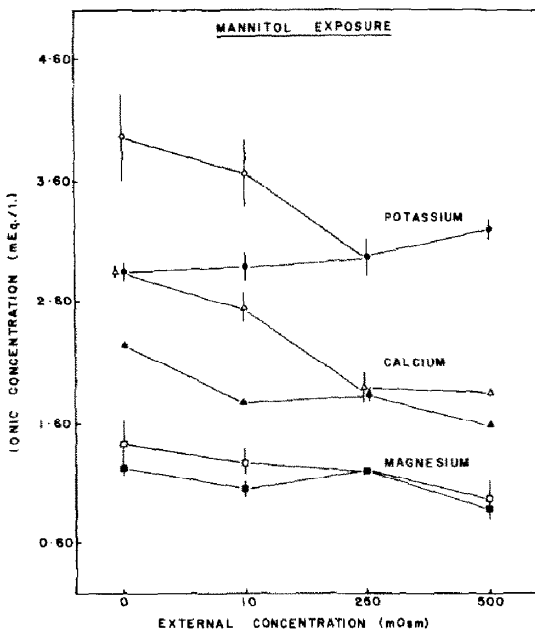


Fig. 6. Relationship between maximum plasma potassium, calcium and magnesium concentrations (unshaded symbols) during exposure of rainbow trout to various mannitol concentrations and level after 7 days (shaded symbols). Bars  $\pm$  2 S.E.,  $n = 5$ .

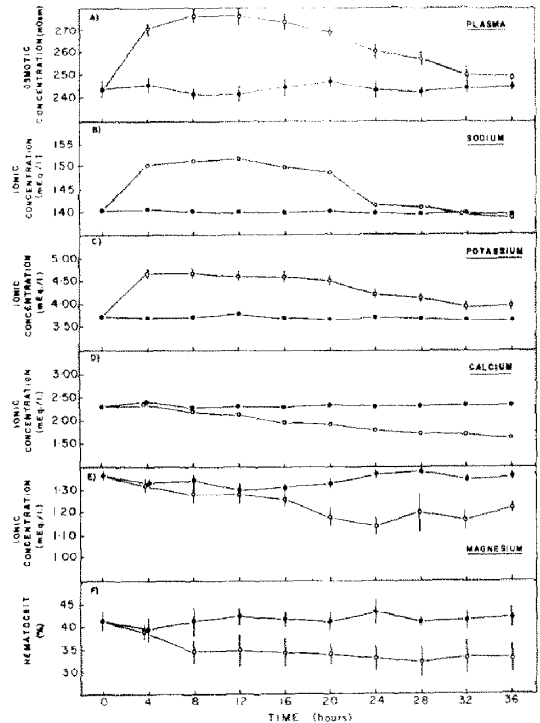


Fig. 7. Responses of rainbow trout exposed to freshwater and sodium chloride. (A) Relationship between plasma osmotic concentration and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). (B) Relationship between plasma sodium concentration and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). (C) Relationship between plasma potassium concentration and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). (D) Relationship between plasma calcium concentration and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). (E) Relationship between plasma magnesium concentration and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). (F) Relationship between hematocrit and time for fish exposed for 36 hr to freshwater (shaded symbols) and 200 mOsm sodium chloride (unshaded symbols). Bars  $\pm$  S.E.,  $n = 5$ .

The urine flow rate and composition also varied with exposure time and concentration. Urine flow decreased after implantation of catheters and reached stable rates as measured in the control (fresh water) group by the fourth day [Fig. 8(A)]. This is slightly longer than the 3 day period previously reported by Kostecky (1979). These data suggest that the fish recovered from stress due to catheter implantation by Day 4 (McKim, 1968).

The rate of urine production decreased over time at the higher external concentrations [Fig. 8(A, C)]. However, the reduction was less in fish exposed to mannitol [Fig. 8(B)] than it was in those tested in sodium chloride [Fig. 8(D)] solutions.

Osmotic concentration of the urine showed very little change in fish exposed to any external concentration, either with mannitol or sodium chloride. The urine osmotic concentration did not exceed 3 mOsm (the minimal resolution level of the analyses) in any

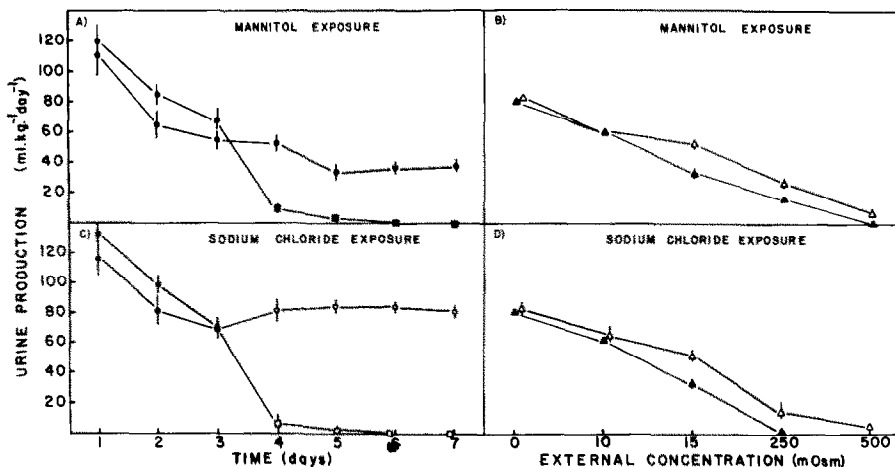


Fig. 8. Typical responses of rainbow trout exposed to mannitol and sodium chloride. (A) Exemplary relationship between urine production and time for fish exposed to ambient concentrations of 500 mOsm mannitol (shaded squares) and freshwater (shaded circles). (B) Relationship between maximum urine production (unshaded symbols) during exposure of fish to various ambient mannitol concentrations and levels after 4 days (shaded symbols). (C) Exemplary relationship between urine production and time for fish exposed to ambient concentrations of 500 mOsm sodium chloride (unshaded squares), deionized water (unshaded circles) and freshwater (shaded circles). (D) Relationship between maximum urine production (unshaded symbols) during exposure of fish to various ambient sodium chloride concentrations and levels after 4 days (shaded symbols) Bars  $2 \pm$  S.E.,  $n = 5$  except (C)  $n = 3$  for 500 mOsm sodium chloride and (D)  $n = 3$  for 500 mOsm sodium chloride.

group of fish. This finding is contrary to other reports by investigators, who have shown that urine concentration of some fish varies drastically in response to changes in the external salinity (Lahlou, 1967). Only exemplary data of the major trends are presented for the purpose of this paper. The reader is directed to Kostecki (1981) for the complete data set.

#### DISCUSSION

The fish were experimentally exposed to two sets of situations; changes in the osmotic gradient with no change in the ionic gradient (mannitol) and simultaneous changes in both the osmotic and ionic gradients (sodium chloride and deionized water). The only ionic gradient that was manipulated was the sodium (and chloride) gradients; potassium, calcium and magnesium being held constant in the deionized water, sodium chloride and mannitol media.

However, despite only two ambient variables being altered, fish responses varied over time and according to the ambient concentration above and below plasma osmotic concentration. These time dependent responses occurred over a short crisis period preceding establishment of new stable conditions. Concentration-related responses depended on whether the medium was more or less concentrated than the plasma. For example, a hyper-osmotic external mannitol solution induced an increased plasma osmotic concentration, whereas an equal hypo-gradient had no such effect. Similarly, greater effects on plasma ions occurred with hyper-ion-osmotic solutions than with hypo-solutions. Therefore, each of these responses, as they relate to hypo- and hyper-conditions are discussed separately.

#### Short-term experiments—crisis period

The observations on fish exposed to changes in ionic and osmotic gradients (sodium chloride) ex-

hibited a "crisis" period (Bath and Eddy, 1979) when large changes occurred in the plasma (24–48 hr after exposure). This period was therefore examined in detail by the short-term experiments. As expected maximum values for plasma osmotic, potassium and sodium concentrations and hematocrit occurred during the crisis period [Fig. 7(A–C, F)]. The crisis period was followed by a stabilization period, when slower physiological changes occurred. The stabilization period appeared to be of a shorter duration than previously reported for smaller trout (Bath and Eddy, 1979). Stabilization appeared to be almost complete with respect to plasma osmotic, sodium and potassium concentrations after 36 hr of exposure. However, no crisis period and, hence, no stabilization period for plasma calcium and magnesium concentrations occurred [Fig. 7(D, E)].

A fish in freshwater is confronted by problems of water influx and salt loss because of the nature of the osmotic and ionic gradients. The addition of sodium chloride up to 200 mOsm was below the isosmotic level of the fish ( $244 \pm 5$  mOsm). At this concentration there was  $99 \text{ mEq l}^{-1}$  sodium in solution as compared to  $141 \text{ mEq l}^{-1}$  sodium in the fish plasma. Thus, the external concentration only reduced the osmotic and sodium gradients, but did not reverse them with respect to the control fish. This change would be expected to reduce regulatory problems, but, in practice, the increase in plasma osmotic and sodium concentrations indicate greater imbalance in plasma levels. Therefore, ionic and osmotic regulatory mechanisms appear to lag in response to the changing external concentrations.

Urine flow is the primary source of water regulation in freshwater fish and it quickly decreased in fish exposed to elevated sodium chloride levels [Fig. 8(D)]. Ionic losses are minimized by active re-absorption in the kidney and losses are replaced by

active uptake at the gills. The plasma sodium concentration and increases in plasma osmotic concentration would be explained if there was a lag time in compensation for the new gradient. The active transport mechanisms would proceed at the same rate and in the same direction as in freshwater after the external sodium concentration was increased. This lag time would be the "crisis" period.

The gradual decrease in plasma calcium and magnesium concentrations may be due to their role in passive, long-term ion-osmoregulation through their involvement in permeability changes. Lowenstein *et al.* (1967) have shown that the permeability of membrane surfaces in *Chironomus* salivary glands depends on the calcium and magnesium ion concentrations. It has been known for some time that calcium ions may contribute to a decreased permeability and reduce water and ion fluxes in some fish (Kirschner, 1969; Motais *et al.*, 1965; Potts and Fleming, 1970). Smith (1970) showed that *Nereis diversicolor* was unable to osmoregulate in calcium-free brackish water. Eels are unable to osmoregulate in seawater and die of dehydration in calcium-free seawater (Sharatt *et al.*, 1964).

The exact nature of the effect of calcium on permeability is not known. It has been suggested that calcium reduces the hydration of polar organic molecules by reducing the repulsion between fixed anions. This results in a closer packing of the organic molecules and, hence, a reduction in permeability. It has also been suggested that the permeability of the body surface depends not only on the permeability of the constituent cells but also on the permeability of the intercellular cement (Shanes, 1960; Houston, 1964; Lowenstein *et al.*, 1967). Calcium stabilizes the intercellular cement making it less permeable.

The decrease in hematocrit in trout exposed to sodium chloride probably occurred as a result of the increase in plasma osmotic concentration. This increase would cause water to be osmotically withdrawn from the erythrocytes, reducing their size. Thus, the size reduction of individual erythrocytes would decrease the hematocrit.

#### *Long-term hyper-osmotic and hyper-ion-osmotic experiments*

Fish exposed to hyper-osmotic (mannitol) and hyper-ion-osmotic (sodium chloride) solutions showed increases in plasma osmotic concentration [Fig. 1(B, D)]. The osmotic gradient for fish exposed to these concentrations is such that the increase in plasma osmotic concentration is likely due to an osmotic dehydration of the plasma. However, osmotic dehydration would cause hemoconcentration; i.e. an increase in plasma ionic concentration. The absence of an increase in plasma ions for fish exposed to hyper-osmotic solutions must therefore be associated with transport of these ions either into storage compartments or out of the animal. This transport would require energy which could account for the significant increase in oxygen consumption in fish exposed to hyper-osmotic mannitol solutions (Kostecki, 1981). The constant ion concentration, but increased osmotic concentration suggests that the increase in plasma osmotic concentration was likely due to large non-diffusible molecules.

In contrast, the increase in plasma osmotic concentration for fish exposed to hyper-ion-osmotic solutions can be largely attributed to increased plasma ions. If a concomitant increase in anions is assumed to maintain electrical parity, sodium, potassium, calcium and magnesium increases account for 90% of the plasma osmotic concentration increase in fish exposed to 500 mOsm sodium chloride. Thus, of the 44 mOsm increase in plasma osmotic concentration about 40 mOsm was due to ions and presumably 4 mOsm to non-diffusible molecules in these fish. This, of course, is an oversimplification since the ionic concentrations contributing to the osmotic concentration vary due to a number of factors, for example, Gibbs-Donnan equilibria and, ultimately, their activity. Thus the calculations serve only as qualitative estimates of the osmotic contribution of large non-diffusible molecules.

The increase in plasma osmotic concentration in fish exposed to the hyper-ion-osmotic solutions is presumably due to the plasma sodium concentration exceeding the excretion rates plus some hemoconcentration as in the hyper-osmotic mannitol concentration.

Ion transport in the adaptation of fish to high salinities has been shown not only to include extra-renal monovalent ion excretion and renal bivalent ion excretion, but also shifts in tissue and extracellular fluid ionic content (Miles *et al.*, 1968; Bath and Eddy, 1979). The shift of ions to body compartments (e.g. interstitial fluid) other than the plasma has been largely ignored in the analyses of energy expenditure for ionic regulation. However, the interstitial fluid comprises almost 7 times the volume of plasma in freshwater teleosts (Thorson, 1961). The relative magnitude of the interstitial volume could dampen plasma ionic concentration changes but substantially add to energy expenditure during adaptation of fish to high salinities. The processes of excretion and shifting both involve active transport.

Plasma sodium concentrations were not the only changes to occur under hyper-osmotic and hyper-ion-osmotic conditions. Plasma calcium and magnesium concentrations of fish exposed to these conditions also declined (Figs. 4, 6), with no differences between mannitol and sodium chloride. The decrease in these ion levels was not due to excretion because urine ionic levels were unchanged and the rate of urine output declined. Therefore, the divalent ions must be regulated by tissue storage. The decrease in plasma calcium and magnesium concentrations may be due to their involvement with permeability changes. However, the similar decrease of these ions in response to osmotic as well as ion-osmotic changes suggests that if these ions are involved in permeability changes, it is with respect to water permeability rather than sodium and chloride ion permeability.

Plasma potassium concentrations remained relatively constant in fish exposed to various osmotic concentrations, suggesting that potassium is relatively inactive in osmotic regulation. However, plasma potassium of fish exposed to hyper-ion-osmotic concentrations followed similar trends to sodium, increasing immediately after exposure but returning to pre-exposure levels by Day 7 (Fig. 3). Since the plasma potassium concentrations of fish

exposed to ion-osmotic media paralleled plasma sodium, there may be some association of these two ions with the ion regulatory mechanisms.

Urine production decreased at hyper-osmotic and hyper-ion-osmotic concentrations and no increase occurred in urine concentration. Therefore, it appears that the kidney is not a major monovalent regulatory route at these concentrations. In contrast, some investigators have indicated that fish exposed to high salinities produce urine of high osmotic concentration (Lahlou, 1967). However, this was probably due to increased urine divalent ion concentration. These experiments were performed in seawater where all ions were increased simultaneously so that divalent influx would have been increased in addition to sodium chloride.

#### *Long-term hypo-osmotic and hypo-ion-osmotic experiments*

The hypo-osmotic and hypo-ion-osmotic situations present different problems and regulatory mechanisms. Excess water excretion together with bivalent ion regulation (conservation) occur in the kidney. Monovalent ion regulation (uptake) occurs at the gills. The large water gradient associated with both hypo-ion-osmotic concentrations (mannitol and sodium chloride) as well as freshwater concentrations is reflected in high production of copious urine. The urinary osmotic concentrations of fish exposed to these concentrations were below detectable levels, consistent with the low urinary ionic concentrations. Reabsorption through active transport would be required to maintain the low urinary ionic concentrations and, since urine is produced at a high rate, reabsorption could require substantial energy expenditure (Kostecki, 1981).

Thus it appears that ionic and osmotic regulation may involve ion excretion and transfers between body compartments. These transfers occur shortly (12 hr) after exposure to different osmotic and ion-osmotic concentrations and would be associated with energy expenditure for regulation. It also appears that calcium and, perhaps, magnesium may be involved in osmotic regulation and in terms of ionic regulation the kidney is of minor importance.

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