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Chemical Properties of the Saccular Endolymph in the Rainbow Trout, *Salmo gairdneri*

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Abstract

Saccular endolymph was analyzed for electrolyte composition and total CO_2 content in the rainbow trout, *Salmo gairdneri*. The endolymph contained a potassium concentration of 26 times as high as in serum. Sodium concentration was slightly lower while chloride concentration was slightly higher in the endolymph than in serum. The calcium and magnesium concentrations were about 60% of those of serum. Total CO_2 (mostly HCO_3^-) and pH values of the endolymph considerably surpassed those of serum and varied diurnally, almost paralleling between the two fluids. Total CO_2 indicated high and low levels during light and dark photoperiods respectively, and pH changed conversely. Acetazolamide, an inhibitor of carbonic anhydrase, induced increases in total CO_2 and calcium levels, and a decrease in pH in saccular endolymph and serum. A single injection of estradiol-17β resulted in marked hypercalcemia, while calcium concentration in the endolymph remained unaffected.

The inner ear of teleosts is composed of a membranous labyrinth which differs greatly from that of mammals in the complete absence of a cochlea. The labyrinth is generally divided into a pars superior (semicircular canals and utriculus) and a pars inferior (sacculus and lagena). The pars superior is concerned with the maintenance of equilibrium and the pars inferior functions as a receptor of gravity and sound (Lagler *et al.*, 1962; Lowenstein, 1971).

In rainbow trout (non-Ostariophysi), the sacculus is a closed sac filled with endolymph and contains a large calcified stone, or otolith (sagitta). The otolith, at least in part, is directly in contact with the lymph and probably functions to generate microphonic potential by vibrating and stimulating sensory hair cells in the macula. The otolith also serves to date the fish because daily and seasonal growth marks are recorded on its surface. Therefore, it would be important to elucidate physicochemical properties of the endolymph for otolith maintenance as well as auditory physiology.

In mammals, the production and electrochemical composition of inner ear (cochlear) endolymph have been studied by many researchers, and the stria vascularis is considered to be a site of fluid production. The endolymph is well defined in composition by high potassium and low sodium, showing intracellular-like composition (Sterkers *et al.*, 1984). In fish, however, little is known concerning these aspects of endolymph physiology. As far as the authors are aware, the only two studies on sodium and potassium determinations in the saccular endolymph of teleosts are by Enger (1964) and Fänge *et al.* (1972), who showed that sodium concentration in the fluid was not as low as in mammals.

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In the present study, saccular endolymph was analyzed for electrolyte composition (Na, K, Cl, Ca, Mg and H) and total CO_2 content, and the results were compared with those of serum in rainbow trout. Hydrogen ion concentration and CO_2 content were further examined for diurnal variations. Effects of acetazolamide and estradiol-17β on endolymph composition were also examined.

Material and Methods

The rainbow trout, *salmo gairdneri*, were obtained from a commercial dealer and reared with running water at about 14°C in the outside ponds of our laboratory. They were fed fish fool pellets once a day in the morning and weighed 85-330 g. When their ovaries were autoptically found to be in a maturing condition, data on calcium determination were omitted.

After fish were netted from the ponds, blood was quickly collected from the caudal artery and vein by cutting the tail of the fish and placing it in plastic tubes. The collected blood was centrifuged and the separated serum was stocked at −30° C for 18–20 hr until analyzed for concentrations of: sodium, potassium and calcium by flame photometry using an atomic absorption spectrophotometer (Hitachi, 518); magnesium by atomic absorption spectrophotometry; and chloride by a chloride meter (Jooko, C-50).

Blood samples for CO_2 determination were collected anaerobically by inserting the needle-like tip of a fine glass capillary containing liquid paraffin (1 cm in thickness) into the caudal artery and introducing blood into the capillary. Then the other side of the capillary was quickly sealed with the paraffin. After centrifugation, the serum was analyzed for total CO_2 content by the Natelson's gas microanalyzer (Kayagaki, Ltd.). This technique was found to minimize the release of blood CO_2 to air and to maintain a constant gas level for at least 2 hr after collection and centrifugation.

For the pH determination of blood, the following technique was devised successfully: the needle-like tip of a capillary (3 mm in diameter) was inserted into the caudal artery and blood was introduced into it. Then the capillary was set up by putting its fine tip into cray seal and a pH microelectrode of 1.2 mm in diameter (Microelectrode, Inc.) connected with a pH meter (Hitachi-Horiba, F7) was inserted into the capillary, inducing the overflow of the air-contacted blood. Using this method, blood pH remained unchanged for at least the first 60 sec.

Saccular endolymph was collected immediately after blood collection by inserting the needle-like tip of a capillary tube into the sacculus and by sucking lightly. Great care was taken to avoid contamination with other body fluids (Mugiya, 1964). Liquid paraffin was used to collect the fluid samples anaerobically for CO_2 determination. As the volume of the endolymph obtained from one individual was not enough for each determination, the fluid samples from 3-5 individuals were pooled and analyzed.

The pH of the endolymph was measured directly by inserting the microelectrode into the sacculus. In this method, pH increased by 0.1 unit in 60 sec during the measurement, mainly due to the release of CO_2 from the fluid to air. A value in the first 10 sec was adopted.

Acetazolamide (Lederle), an inhibitor of carbonic anhydrase, is known to reduce the growth rate of fish otoliths (Mugiya, 1977; Mugiya *et al.*, 1979) and to alter the endocochlear potential in rats (Sterkers *et al.*, 1984). Fish were given an intraperitoneal injection of this drug at a dose of 100 mg/kg and 24 hr later were examined for its effects on pH, total CO_2 and calcium levels in serum and saccular endolymph. The control was given saline only.

Estadiol-17β (Sigma), which is known to induce hypercalcemia, was injected intraperitoneally at a dose of 10 mg/kg, suspending it in sesame oil, and serum and saccular endolymph were sampled 2 weeks after injection to analyze their calcium concentrations. The control was given sesame oil only.

Carbon dioxide and pH were determined diurnally at 4 or 5 times (1000, 1600, 2200, 0400 and 0700 hr) throughout a 24 hr period. Sampling for the other variables was made once a day between 1000 and 1100 hr. Calcium was determined on 3 different days and the data were pooled.

Results

Saccular endolymph is unique in chemical composition, showing a potassium concentration of 26 times as high as in serum (Table 1). Sodium concentration was slightly lower while chloride concentration was slightly higher in the fluid than serum. The calcium and magnesium concentrations were about 60% of those of serum.

The endolymph was also differentiated from serum by higher values in total CO_2 content and pH (Table 1), and these two parameters changed diurnally in both fluids (Figs. 1 and 2). In serum, total CO_2 showed the highest level in the morning (1000 hr), followed by a steady decrease to the minimum at the following dawn (0400 hr). It changed by 30% during a 24 hr period and this change was statistically significant at $P=0.05$. In saccular endolymph, the content of total CO_2 was higher by 38-48% (42% on the average) than that of serum at any examination time, and changed diurnally in a pattern similar to the changes in the serum, showing the maximum and minimum levels at 1000 and 0400 hr respectively. It

Table 1. Electrolyte composition (mEq/l), total CO_2 content (vol %) and pH of saccular endolymph and serum in rainbow trout.

	Serum	Endolymph
Na	149.52±0.39 (12)*	139.43±1.78 (6)[a]
K	1.28±0.09 (12)	33.81±1.68 (7)[a]
Cl	121.13±1.32 (6)	132.05±1.08 (6)[a]
Ca	5.14±0.12 (24)	3.20±0.15 (13)[a]
Mg	1.85±0.07 (12)	1.06±0.04 (7)[a]
CO_2	32.64±1.09 (14)	47.47±2.96 (4)[a]
pH	7.56±0.02 (23)	8.06±0.04 (25)[a]

* Mean±SE (No. of fish for serum or determinations for endolymph).

[a] Significant at $P=0.001$ for serum.

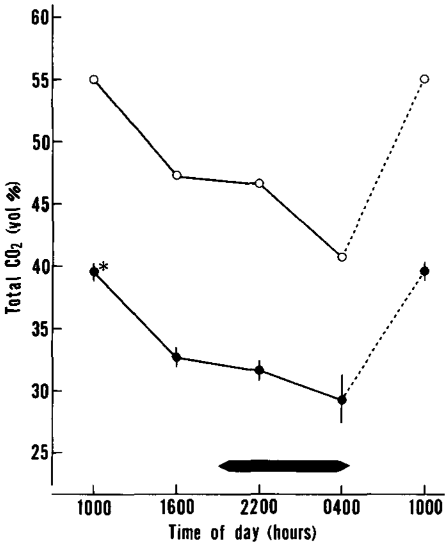

Fig. 1. Diurnal variations of total CO_2 content in saccular endolymph (○) and serum (●) in rainbow trout. Each plotted point represents mean±SE of 2-4 fish for serum and the value of a single determination of the endolymph pooled from 3-5 fish. Dark horizontal bar indicates nocturnal and twilight periods.
* $P<0.05$ for 0400 hr.

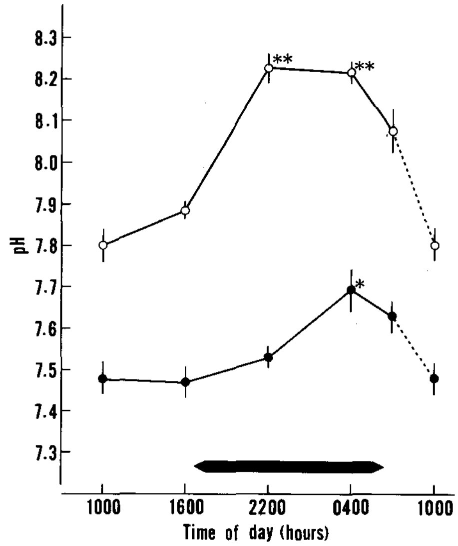

Fig. 2. Diurnal variations of pH values in saccular endolymph (○) and serum (●) in rainbow trout. Each plotted point represents mean±SE of 4-6 fish. Dark horizontal bar indicates nocturnal and twilight periods.
* $P<0.002$ for 1000 and 1600 hr.
** $P<0.001$ for 1000 hr.

Table 2. Effects of acetazolamide and estradiol-17β on the levels of total CO_2 (vol %), pH, and calcium (mEq/l) in saccular endolymph and serum in rainbow trout.

			Serum	Endolymph
Acetazolamide	CO_2	Cont	32.52±0.63 (20)*	52.51±2.60 (4)
		Exp	36.42±1.23 (10)[d]	71.69±6.81 (3)[a]
	pH	Cont	7.57±0.05 (7)	8.13±0.07 (5)
		Exp	7.26±0.08 (6)[c]	7.81±0.10 (4)[a]
	Ca	Cont	5.10±0.10 (7)	2.70±0.03 (2)
		Exp	5.41±0.06 (8)[b]	2.99±0.02 (2)[b]
Estradiol	Ca	Cont	5.56±0.22 (5)	2.95
		Exp	19.72±1.05 (5)[e]	2.65

* Mean±SE (No. of fish for serum or determinations for endolymph).
[a] Significant at $P=0.05$, [b] significant at $P=0.02$, [c] significant at $P=0.01$,
[d] significant at $P=0.005$, [e] significant at $P=0.001$.

showed a 30% variation during a 24 hr period. Serum pH showed a distinct diurnal pattern which corresponded well to the photoperiod of light and dark phases (Fig. 2). After sunset, the pH increased gradually to the peak at dawn, followed by a rapid decrease to a low level of daylight time. These up-and-down changes were both significant at $P=0.002$. The pH of saccular endolymph was always higher by 0.3–0.7 unit (0.5 unit on the average) than that of serum and changed diurnally ($P<0.001$), showing the low and high values at light and dark photoperiods respectively. The maximum pH was obtained from 2200 to 0400 hr. After dawn, the pH dropped rapidly and the minimum level was reached at 1000 hr, followed by a slight increase toward 1600 hr.

When fish were given acetazolamide, they became blackish in color and inactive in movement. Twenty-four hours after injection, the level of total serum CO_2 increased by 12% ($P<0.005$) over the control level (Table 2). Such acetazolamide-induced hypercapnia also occurred in the saccular endolymph ($P<0.05$). Serum and endolymph pH decreased significantly by about 0.3 unit each in the experimental group ($P<0.01$ for serum and $P<0.05$ for endolymph), while calcium levels in both fluids increased by 6–11% ($P<0.02$) (Table 2).

A single injection of estradiol-17β, which appeared to have no effect on the behavior of the fish, resulted in a 3.5-fold increase in serum calcium 2 weeks after injection ($P<0.001$), but no effect was found in the level of endolymph calcium (Table 2).

Discussion

Although the stria vascularis is known to be a site for production of inner ear endolymph in mammals, fish lack this structure and nothing is known about where and how the endolymph is produced. As the sacculus of the present fish (nonostariophysan form) is a closed sac, the endolymph is probably a product secreted through some specialized sites in the sac epithelium. Saito (unpublished) recently found an oval path of mitochondria-rich cells in the lateral wall of the sacculus and has suggested an involvement of the cells in the production and modification of saccular endolymph in tilapia.

The electrolyte composition of saccular endolymph was characterized by a high content of potassium. As rainbow trout have a rather low concentration of serum potassium, which agrees with the results by Enger (1964), a ratio of 26 was obtained in potassium concentration between the endolymph and the serum. The functional significance of high potassium concentration in the labyrinthine fluids may be related with electrophysiological events taking place in the mechanoreceptors (Flock, 1965).

Erulkar and Maren (1961) found that the inner ear of cats contained a very high activity of carbonic anhydrase and suggested that the enzyme played an important role of potassium transport into the labyrinthine fluids. In the saccular tissue of rainbow trout, however, the activity of carbonic anhydrase was rather weak and shown to be involved in otolith formation (Mugiya *et al.*, 1979). Sodium-potassium ATPase is another candidate for energizing an electrogenic potassium pump to maintain the intracellular-like composition of endolymph (Sterkers *et al.*, 1984).

Another feature of saccular endolymph is the considerably high content of total

CO_2 as well as the high value of pH. In the present measurements, total CO_2 was determined as the sum of CO_2 dissolved as gas, H_2CO_3, HCO_3^- and $CO_3^=$, and the proportion of each component remains unknown. However, according to the Henderson-Hasselbalch equation, high alkalinity in the fluid suggests that the fluid contains alkali reserve (HCO_3^-) in a high rate, which would provide a favorable micro-environment for otolith maintenance. The presence of carbonic anhydrase in the saccular tissue is probably responsible for maintaining a high concentration of alkali reserve in the fluid.

Serum total CO_2 varied diurnally, showing the minimum and maximum levels at 0400 and 1000 hr respectively. As sunrise was at 0426 hr on the day of the experiment, the rapid increase in the CO_2 content is probably due to an increase in activity of the trout after sunrise. This is supported by the observation that the fish stayed still during dark periods while they were active during light periods. Activity results in the high production of CO_2 and some metabolic acids (Wood *et al.*, 1983). Serum pH also varied according to the photocondition (sunrise: 0605 hr), showing high and low values during dark and light periods respectively. Accordingly, serum pH appeared to change in relation to fish activity.

Total CO_2 content and pH in saccular endolymph showed diurnal variations corresponding closely to those of serum. As the volume of the enolymph is infinitesimal compared with that of serum and the membrane permeability of CO_2 is high, these changes in the endolymph might be passively induced, at least in part, by the respective changes in the serum.

Acetazolamide significantly affected total CO_2, pH and calcium levels in serum. This drug is known to inhibit the activity of carbonic anhydrase, which plays an important role of CO_2 excretion in the gills of fish. Therefore, the inhibition of this enzyme will induce the accumulation of metabolized CO_2, resulting in the drop of serum pH by the production of carbonic acid. Acidosis is known to mobilize skeletal calcium and to increase plasma calcium levels in fish and other vertebrates (Ruben and Benett, 1981). This will explain the present increase in serum calcium concentration in the acetazolamide-treated group.

Acetazolamide induced similar changes in these three variables of saccular endolymph. These changes appear to be mostly due to the secondary effects of the changes in the serum. As far as total CO_2 content is concerned, however, the local effect of the drug is expected because of the presence of carbonic anhydrase in the saccular tissue (Mugiya *et al.*, 1979). The enzyme catalyzes the conversion of CO_2 to HCO_3^- and thereby produces $CO_3^=$ actively, which is eliminated by deposition on the otolith as $CaCO_3$. Therefore if this process is reduced by acetazolamide, the carbonate deposition will be slowed down and the local level of total CO_2 will be increased to some extent. *In vivo* and *in vitro* inhibitory effects of the durg on otolith calcification were reported in goldfish (Mugiya, 1977) and rainbow trout (Mugiya *et al.*, 1979).

Although a single injection of estradiol resulted in a marked increase in calcium concentration of serum, that of the saccular endolymph remained unaffected. This hormone is known to increase the plasma level of protein-bound calcium with no effect on that of ionized calcium. In the case of the acetazolamide-induced hypercalcemia, which is probably due to an increase in ionized calcium, the level of endolymph calcium also increased. Therefore, endolymph calcium is probably in some equilibrium with the diffusible form of serum calcium, though this does not necessarily mean that endolymph calcium constitutes a mere ultrafiltrate of ionized or diffusible calcium in the serum (Mugiya, 1974). Further studies are needed to elucidate metabolic aspects of electrolytes in labyrinthine fluids in teleosts.

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