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# 1,25-Dihydroxyvitamin D in Male, Nonspawning Female, and Spawning Female Trout (41061)

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*Abstract.* The concentration of circulating 1,25-dihydroxyvitamin D was measured by means of a specific radioreceptor assay in male, nonspawning female, and spawning female trout. Male trout showed serum levels of $1,25(OH)_2D$ which were comparable to those of weanling rats and infant children and higher than levels found in female trout. 1,25-dihycrosyvitamin D levels in nonspawning and spawning female trout were comparable, despite significant higher levels of circulating calcium and phosphate in the spawning trout. The study documents the existence of 1,25-dihydroxyvitamin D in the blood of teleost fish for the first time.

The reproductive cycle in fish is characterized by extensive resorption of calcium depots (1–3), and progressive elevations in serum calcium concentrations, the latter reflecting primarily elevations in the protein bound calcium moiety (3, 4). To date, the hypercalcemia of spawning fish has been attributed primarily to an increased secretion of estrogen (4, 5). 1,25-Dihydroxycholecalciferol ($1,25(OH)_2D_3$) has been shown to induce osteoclastic bone resorption in the eel (6), and the 25-hydroxycholecalciferol (250HD) precursor substrate circulates in both the eel (6) and bony fish (7, 8). Since $1,25(OH)_2D$ plays a pivotal role in modulating the mobilization of calcium resources in vertebrate species during the reproductive cycle (9, 10), we investigated the possibility that this vitamin D metabolite functioned in a similar fashion in spawning teleost fish.

**Material and Methods.** Fresh male and female rainbow trout, *Salmo gairdneri,* 1 year of age were purchased from a nearby fishery and blood samples obtained by cardiac puncture without anesthesia. Following centrifugation at 2000 rpm for 20 min at 4° the serum was separated. Aliquots were subjected to measurements for total calcium utilizing the Turner Fluorometer Model II and phosphate (as inorganic phosphorus) was determined colorimetrically (11). For $1,25(OH)_2D$ quantitation, 2 ml of sera was supplemented with 2000 dpm of $1,25(OH)_2[-^3H]D_3$ (110 Ci/mmole) to determine yield of the isolation and the sterols extracted with acetone. Extraction was carried out by shaking vigorously with 5 vol of distilled acetone for 20 min, removing the residual protein by centrifugation and evaporating the supernatant with nitrogen. When the volume was reduced to 1–2 ml of aqueous suspension, the sterols were extracted with 15 ml diethylether and the aqueous remnant was discarded. The diethylether phase was dried under nitrogen and resolubilized in the appropriate organ solvent for chromatographic purification. Purification of $1,25(OH)_2D$ was initially performed on silicic acid and LH-20 columns (12). The $1,25(OH)_2D$ was first eluted from the silic acid column with acetone after washing with 75% hexane in ether. Further purification on LH-20 was accomplished by elution with 65% $CHCl_3$ in hexane. Final isolation of the $1,25(OH)_2D$ was obtained using high performance liquid chromatography (hplc). The hplc was performed with a Dupont 830 liquid chromatography fitted with a Dupont Zorbax-Sil-850 column (25 cm × 4.6 mm). Samples were injected with a Rheodyne Model 70-10 sample injection valve and Model 70-11 loop-filter port with a 200 μl sample loop. The system was equilibrated and run with 15% isopropanol in hexane at a flow rate



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of 1 ml/min. $1,25(OH)_2D_3$ eluted between 14 and 17 min from the time of injection and was not significantly resolved from $1,25(OH)_2D_2$ under these conditions (13). The eluted fraction was dried with nitrogen and solubilized in 400 $\mu$l ethanol for yield determination and radioreceptor assay. With this purification scheme, yields of $1,25(OH)_2[^3H]D_3$ were 50–70%.

Radioreceptor assay, as originally described by Brumbaugh *et al.* (14), was performed in triplicate employing a number of recent modifications (12). The assay involves a specific cytosol–chromatin receptor preparation from rachitic chick intestine; the system is saturable and binds $1,25(OH)_2[^3H]D_3$ with a high affinity ($Kd = 2 \times 10^{-10}$ $M$). Competitive binding with authentic nonradioactive $1,25(OH)_2D_3$ (M. Uskokovic, Hoffman–LaRoche) is sensitive to 1.0 pg/tube, with a binding efficiency of 25% and an interassay variation of 10–15% (12). Using this radioreceptor assay, normal concentrations of circulating $1,25(OH)_2D$ are 33 ± 6 (SD) pg/ml in 45 adult U.S. humans on nutritionally adequate diets.

**Results and Discussion.** As illustrated in Table I, not only does $1,25(OH)_2D$ circulate in teleost fish but it does at concentrations which are consistently greater than those of normal humans (14). The values are, however, comparable with those of weanling rats (12) and human infants (15). Although fish are known to be a rich source of antirachitic factor(s), to our knowledge this is the first evidence for the existence of circulating $1,25(OH)_2D$ in this species. Henry and Norman (16) have detected kidney 25-hydroxyvitamin D-1$\alpha$-hydroxylase in both fresh and salt water fish, indicating that the enzymatic capability of producing $1,25(OH)_2D$ is present. Nahm *et al.* (8) have also recently shown that 250HD occurs in reasonable concentrations in fish, thus providing the appropriate substrate for $1,25(OH)_2D$ biosynthesis. Nevertheless, previous attempts to detect $1,25(OH)_2D$ in fish with labeled precursors have been unsuccessful (17, 18).

The fact that $1,25(OH)_2D$ blood levels are higher in male trout than either nonspawning or spawning female trout (Table I), is still unexplained. These findings are, however, reminiscent of the data of Nahm *et al.*, who noted that blood and hepatic levels of 250HD in male trout were significantly higher than those of nonspawning female trout (8). The accumulated data are also consistent with the conclusion that, in contrast to results obtained with other species (9), the hypercalcemia which attends the reproductive cycle in fish is not dependent on alterations in circulating $1,25(OH)_2D$ (Table I).

Although it is conceivable that our radioreceptor assay is detecting a sterol which is very similar but not identical to $1,25(OH)_2D$, this possibility is unlikely because of the combined use of hplc purification and subsequent specific binding assay. Thus, any $1,25(OH)_2D$-like molecule would have to both migrate uniquely with $1,25(OH)_2D$ in the HPLC utilized in this study, and also compete efficiently in the highly selective chromatin receptor system.

TABLE I. SERUM TOTAL CALCIUM, PHOSPHATE, AND $1,25(OH)_2D$ IN FEMALE AND MALE TROUT[a]

| | $(n)$[b] | Calcium (mg/dl) | Phosphate (mg/dl) | $1,25(OH)_2D$ (pg/ml) |
|---|---|---|---|---|
| Male | (14) | 11.2 ± 0.4 (9.2–13.8) | 10.9 ± 0.4 (9.7–15.9) | 130 ± 21 (43–266) |
| Female | (10) | 12.3 ± 0.3 (10.5–13.0) | 11.7 ± 0.7 (8.1–13.3) | 59 ± 7* (37–90) |
| Spawning females | (15) | 17.2 ± 0.7** (14.5–24.3) | 12.6 ± 0.4** (9.8–16.2) | 56 ± 9* (15–165) |

[a] Numbers in parentheses reflect range of observed values.
[b] $(n)$ = number of fish in each instance.
* $P < 0.01$ when compared to male trout.
** $P < 0.01$ when compared to male or nonspawning female trout.

Further work is needed to determine if trout $1,25(OH)_2D$ is the natural $D_3$ metabolite, as occurs in humans and as a glycoside in certain calcinogenic plants, or whether the sterol side chain is slightly altered. $1,25(OH)_2D_2$, which is derived from synthetic vitamin $D_2$ in animals, migrates similarly to $1,25(OH)_2D_3$ on hplc and competes equally with the natural hormone in the radioreceptor assay. Our discovery of a $1,25(OH)_2D$-like sterol in fish obviously still requires chemical and physical verifications. The data to indicate, however, that fish may prove to be an excellent model for studies of calcium and phosphate metabolism as modulated by $1,25(OH)_2D$.


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