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Comparative Uptake of Calcium-45 and Strontium-90 by Wild and Lordotic Guppies.* (23826)

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Investigations concerning metabolism of bone and mineralized tissues have been hampered by lack of suitable experimental animals. Recently, a naturally-occurring mutation in the guppy, *Lebistes reticulatus* (Peters) has been described which forms bone of greater density and higher calcium content (1). The mutation, a single recessive autosomal character, displays marked dorso ventral and lateral curvature of spinal column which has been called "hunchback"(2,3) or "lordosis"(4). Harrison(5) analyzed 3 animals and found that total body calcium is higher in the mutant fish(5). Rosenthal(1) demonstrated that calcium is increased in lordotic spines while muscle calcium concentration is not altered. The calcium:phosphorus ratios of lordotic bone is also greater since the phosphorus content of bones from both strains of fish is the same(1). These data strongly suggest a specific alteration of calcium metabolism is osseous tissues from lordotic animals.

A series of studies with radioactive calcium-45 and strontium-90 was undertaken in order to determine if the metabolic defect leading to an accumulation of calcium in osseous tissues of the lordotic guppy was due to alterations in the rate of incorporation and/or turnover of mineral elements.

Materials and methods. Wild type guppies were obtained from commercial sources and lordotic guppies were raised in this laboratory, (6). The general experimental design, isotope technics and analytical methods have been previously published in detail(1,7-9). In the present comparative studies with wild

* Neutralizing stones were analyzed for calcium and strontium by Crippen and Ehrlich Laboratories, Baltimore, Md. We are grateful for the spectrographic strontium analysis of bone ash performed through the courtesy of Dr. Karl K. Turekian, Yale University. This investigation was performed under contract with the U. S. Atomic Energy Comm.

TABLE I. Uptake of Calcium-45 by the Total Body of Wild-Type and Lordotic Guppies.

Water bath activity, cpm/ml	Wild-type	Lordotic	T	P
	cpm/100 mg fish/day ± S.E.			
1.79×10^2	38.3± 5.5(6)*	27.9± 2.3(5)	1.84	<.10
3.36×10^3	383 ± 27 (3)	333 ± 40 (2)	2.71	<.05
2.77×10^5	27500 ±2530 (5)	17800 ±1550 (5)	4.02	<.01
1.04×10^6	57600 ±3750 (5)	45100 ±6700 (5)	2.54	<.02
	cpm/mg calcium/day ± S.E.			
1.79×10^2	35.9± 5.0(6)	20.5± 1.8(5)	2.89	<.02
3.36×10^3	336 ± 24 (3)	247 ± 3.0(2)	2.87	<.05
2.77×10^5	24100 ±2440 (5)	13200 ±1560 (5)	5.69	<.01
1.04×10^6	50500 ±3270 (5)	33400 ±4050 (5)	5.09	<.01

* No. of animals analyzed in parentheses.

type and lordotic guppies, male fishes of both strains were placed in the same aquarium and treated in an identical manner. Although tap water aged for 24 hours was used in most instances, conditioned water obtained from stock aquaria was used on occasion. Stock aquaria contained neutralizing stones composed of calcium and strontium salts. Spectrographic analysis of the stones indicated a composition of 64.8% CaO and 27.3% SrO. Since tap water in this area contains 23 ppm calcium and less than 1 ppm strontium(10), it was possible to estimate calcium and strontium content of conditioned water from oxalate precipitable material and composition of neutralizing stones.

Results. Calcium concentration of the body of 56 male lordotic guppies and of 76 wild-type males was 1.35 ± 0.03 (S.E.) and 1.14 ± 0.02 (S.E.) % of wet weight respectively, (t for difference = 12.12, P = < 0.01). Although, whole, fat-free spinal columns from lordotic female guppies contain significantly greater quantities of calcium than spinal columns from wild-type fish, spectrographic analysis of 4 pooled samples consisting of spines from 3 female fish indicated strontium concentrations of 0.028% for wild-type and 0.026% for lordotic bone. It is apparent that lordotic mutation is associated specifically with an alteration of calcium metabolism.

Rate of uptake of calcium-45 from tap water by total body is significantly lower for lordotic guppies at all water activities studied (Table I). The lower rate of uptake becomes even more significant when the data are expressed in terms of body calcium content, since lordotic guppy contains more calcium/unit weight than the wild type. When calcium chloride is added to tap water to bring the calcium content to 275 ppm (Table II), this difference is no longer apparent. However, in conditioned water containing intermediate quantities of salts (75 ppm calcium and 20 ppm strontium), the rate of incorporation of calcium-45 by the body of lordotic guppies remains significantly lower than the rate of wild-type guppies. It may be observed, as reported previously(7), that rate

TABLE II. Uptake and Spine:Body Distribution of Calcium-45 for Wild-Type and Lordotic Male Guppies.

Water*	Body activity ± S.E. Wild-type	Body activity ± S.E. Lordotic	Spine:Body ± S.E. Wild-type	Spine:Body ± S.E. Lordotic
	10^3 cpm/100 mg/day			
Tap H_2O	65.2 ± 4.1 (6)†	50.2 ± 6.1 (7)†	1.33 ± .12 (6)‡	2.18 ± .28 (7)‡
Cond. H_2O—{75 ppm Ca, 20 ppm Sr}	27.5 ± 2.5 (5)§	17.8 ± 1.5 (5)§	1.62 ± .04 (5)§	2.25 ± .16 (5)§
Tap H_2O + 275 ppm Ca	7.35± .43(6)	8.20± .86(6)	2.14 ± .53 (7)	1.79 ± .24 (6)

* Water activity ranged between 2.2×10^5 and 2.8×10^5 cpm/ml water.
† P = <0.05. ‡ P = <0.02. § P = <0.01. All other values not significantly different.
No. of fish analyzed in parentheses.

TABLE III. Rate of Turnover of Incorporated Calcium-45 by Wild-Type and Lordotic Male Guppies.

Organ	Days turnover	Turnover rate* ± S.E. Wild-type	%	Lordotic	%
Carcass	0	2.66 ± .25 (5)†	100	1.72 ± .24 (5)†	100
	10	1.96 ± .02 (4)	74	1.39 ± .06 (5)	81
	25	1.50 ± .08 (3)	56	1.16 ± .06 (3)	68
Spine	0	4.01 ± .26 (10)	100	3.93 ± .59 (5)	100
	10	4.96 ± .34 (10)	124	5.11 ± .63 (9)	130
	25	4.66 ± .42 (9)	116	4.54 ± .59 (4)	115

* Activity = 10^4 cpm/100 mg/day. † No. of fish analyzed in parentheses.

of incorporation of isotopes by the body of fishes decreases with increasing salt concentration.

The lower rate of incorporation of calcium-45 into the body of lordotic guppies fails to explain the increased calcium content of the animal, and suggests that the turnover rate of body and bone calcium of lordotic guppies is less than that for wild-type animals. In a 25-day experimental period, the rate of turnover of incorporated calcium-45 by the body (Table III) appears to be somewhat less than that for the wild-type guppy but this difference is not significant. In like manner, the turnover rate for spines is the same for both strains of fish. Since the biological half-lives of calcium in guppies have been calculated to be at least 300 and 600 days for body and spine respectively(7), small differences in turnover rate are difficult to detect. The 25-day experimental period is much too short for definitive studies, but the delicate nature of the lordotic guppy prevents us from maintaining these animals under experimental conditions for longer periods of time. Until further information is available, the data presented in this report suggest that the turnover rate of calcium-45 is less for the lordotic guppy than for the normal.

The apparent alteration of calcium metabolism in lordotic guppies and the chemical and biological similarity of calcium and strontium prompted us to study the uptake of strontium-90 from water by the 2 strains of fish. As shown in Table IV, the rate of uptake of strontium-90 from the water in which they swim is the same for both strains of fish under a wide variety of conditions ranging from tap water of low salt content to conditioned water of high salt content. These results are to be expected since the spinal column from lordotic and wild-type fish contain similar quantities of strontium. The data again emphasize the specific alteration of calcium metabolism in lordotic guppies.

Although the rate of uptake of calcium-45 by the body of lordotic fish is lower than the wild-type, the rate of incorporation of calcium-45 by the spinal column is greater for the lordotic than the normal fish, expressed as the ratio of the uptake of the spine to that of the total body (Table II).

The spine:body relationship is significantly different for the two strains of fish when calcium-45 is taken up from tap water of low salt content or from conditioned water of in-

TABLE IV. Uptake and Spine:Body Distribution of Strontium-90 for Wild-Type and Lordotic Male Guppies.

Water*	Body activity ± S.E. Wild-type	Lordotic	Spine:Body ± S.E. Wild-type	Lordotic
Tap H_2O	17.7 ± .9 (7)	15.8 ± .7 (7)	2.44 ± .26 (7)	2.38 ± .24 (8)
Cond. H_2O—{ 75 ppm Ca, 25 ppm Sr }	4.72 ± .11 (4)	5.12 ± .26 (5)	2.12 ± .13 (4)	1.90 ± .22 (3)
Cond. H_2O—{ 200 ppm Ca	2.36 ± .28 (5)	1.97 ± .19 (4)	2.42 ± .20 (12)	2.40 ± .22 (12)
125 ppm Sr }	2.16 ± .28 (9)	2.21 ± .22 (9)		

* Water activities ranged between 8.6×10^4 and 3.8×10^5 cpm/ml water.
Mean differences between groups not significantly different.
No. of fish analyzed in parentheses.

termediate salt content. At high concentrations of calcium (275 ppm) this difference is eliminated and the ratio becomes the same.

On the other hand, experiments with strontium-90 indicate that the uptake of this element by the body and the spine:body ratios are similar for both strains of fish. It is interesting to note that the strontium-90 spine:body ratio for both strains of fish is similar to the calcium-45 spine:body ratio of lordotic animals. We have previously shown that the proportion of strontium-90 incorporated into bone, relative to the rate of uptake by the total carcass of wild-type guppies is significantly greater than for calcium-45(9). The significance of these data is obscure at the present time and must await further clarification.

These data demonstrate that the hereditary mutation resulting in lordosis is associated with a specific metabolic abnormality involving calcium metabolism. The mutant guppy, easily raised in large numbers under laboratory conditions, may aid in the elucidation of calcium metabolism and bone formation in vertebrate animals, but a complete understanding of this mutant fish must await further, more definitive studies.

Summary. The lordotic mutation in the guppy is associated with a lower accumulation of body calcium and increased incorporation of calcium into bone. Strontium incorporation is the same for both strains of fishes. These studies indicate a specific defect in calcium metabolism which may further an understanding of calcium metabolism and bone formation.

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Hamster Kidney Cell Tissue Cultures for Propagation of Japanese B Encephalitis Virus.* (23827)

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Since 1949, there has been much developmental laboratory work done with monkey kidney and HeLa cell tissue cultures and the more stable viral agents such as polio, Adeno, and ECHO groups. However, these cell systems frequently have failed to yield marked and consistent cytopathogenic effects (CPE) following inoculation with more labile agents as exemplified by arthropod-borne group. Consequently, the search for new tissue culture systems to permit an *in vitro* methodology for these less stable viruses is continuing. The earlier work has been admirably reviewed by Sanders, Kiem and Lagunoff(1). Syverton and Scherer(2) reported serial propagation of Western (WEE), Eastern (EEE), West Nile (WN), St. Louis (SLE), and Japanese B (JBE) encephalitis viruses in HeLa cell tissue cultures noting irregular and inconsistent CPE with SLE and JBE agents. McCollum and Foley(3) reported successful propagation of JBE virus in monkey kidney, chick fibroblasts, and Detroit-6 cell line tissue cultures, but without complete CPE. Banta(4) reported attempts to cultivate JBE, WEE, WN, and dengue (type

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