Strontium Incorporation into Dental Enamel

Abstract. *Rats were raised on diets either rich or poor in strontium. Powder x-ray diffraction patterns suggest that isomorphous substitution of strontium for calcium occurs in the apatite of tooth enamel, and that strontium may form* $Sr_6H_3(PO_4) \cdot 2H_2O$, *a compound hitherto unreported in biologic systems.*

Collin (*1*) has shown that a complete series of homogeneous solid solutions of calcium-strontium apatite can be prepared in vitro. X-ray diffraction methods have shown that incorporation in vivo of trace amounts of strontium in bone, dentin, and enamel of the rat reduces the crystallinity of the mineral phase of these tissues (*2*). X-ray powder patterns of rat-bone ash containing as much as 8 percent strontium produced no evidence of a phase different from calcium apatite, although the unit cell was altered (*3*). We have attempted to determine the effect of strontium, incorporated in vivo, on the powder x-ray diffraction patterns of incisor-tooth enamel of the rat.

Two groups of 21-day-old, male, weanling, Sprague-Dawley rats were fed diets nutritionally adequate and identical except for the calcium, strontium, and phosphorus contents. The strontium-poor diet contained (percent) 0.40 calcium, 0.0002 strontium, and 0.42 phosphorus; the strontium-rich diet contained 0.22 calcium, 0.45 strontium, and 0.41 phosphorus.

The animals were killed at 43 days, and their incisor teeth were removed, cleaned, extracted of fat, and dried at 110°C. Teeth from animals on the strontium-poor regimen were pooled and pulverized to a particle size between 100 and 200 mesh. By use of the flotation method (*4*), enamel was obtained with a density of 2.86 g/cm^3 or greater; its ash content was 97.5 percent on a dry, fat-free basis. The ash contained 36.9 percent calcium, a trace of strontium, and 18.6 percent phosphorus (*5*).

Because strontium causes a less-mineralized tissue to form, the flotation method could not derive strontium-rich enamel from the teeth of animals receiving the strontium-rich diet; therefore lower incisors, one from each of two animals, were used as sources of enamel from rats on the strontium-rich diet.

Two sections of enamel, approximately 3 mm in length and variable in width and thickness, were secured from the labial aspects of the teeth. Portions of the labial and lingual surfaces of the fragments were removed with dental instruments to a depth sufficient to assure that only enamel remained. These fragments encompassed enamel extending from near the junction of the apical and middle thirds to slightly beyond the midpoints of the teeth.

These portions of the incisor teeth were about to be erupted into the oral cavity and may have included enamel that was not completely mineralized. However, enamel from similar regions in normal animals is comprised of crystallites that produce sharp lines on x-ray diffraction films (*6*). One fragment, pulverized, yielded 61.1 percent ash on a dry, fat-free basis; the ash contained (percent) 26.7 calcium, 15.1 strontium, and 20.9 phosphorus. Duplicate x-ray diffraction patterns were made (*7*) from two ball mounts of the pooled strontium-poor enamel pulverized to between 200 and 325 mesh. Two patterns were made from each of four ball mounts of the powdered strontium enamel fragment, and one pattern was made from the intact fragment of strontium-rich enamel.

Twenty-three pairs of arcs were found on the films from the pulverized strontium-rich fragment, but only nine were common to all of the eight patterns. After careful evaluation, six of the nine pairs of arcs appeared to be usable for determining the lattice constants (Table 1). Six pairs of arcs at comparable 2θ-values were selected from the strontium-poor patterns (Table 1). The *a*- and *c*-values were calculated for each kind of enamel and plotted versus the corresponding extrapolation function $\frac{1}{2}(\cos^2\theta/\sin\theta + \cos^2\theta/\theta)$ to determine the corrected lattice constants (*8*). The extrapolated *a*-values of pulverized strontium-poor and -rich enamel were 9.47 and 9.48 Å, respectively; the corresponding *c*-values were 6.86 and 6.91 Å. These results suggest that strontium substitutes for calcium in apatite in vivo, but that the two positions in the unit cell known to be occupied by calcium may not be proportionately available to strontium.

Of forty-one pairs of lines measured on the pattern of the fragment of strontium-rich enamel, several could not be identified as belonging to an apatite. A conservative approach was used in an attempt to identify these unique lines by (i) calculating the *d*-values for a theoretical apatite with an *a*-axis of 9.42 Å and a *c*-axis of 6.88 Å (*9*), (ii) determining all the *d*-values from the interpretable line of the strontium-poor enamel patterns, and (iii) comparing each of these sets of values with those from the pattern of the fragment of strontium-rich enamel. The accepted deviations in *d*-values were based on Zoltai *et al.* (*10*) and expanded to be more inclusive. Table 1 includes *d*-values of the powdered strontium-rich fragment of enamel of the compound $Sr_6H_3(PO_4)_5 \cdot 2H_2O$ (*11*), and of the

Table 1. Comparison of *d*-values (in angstroms) of strontium-poor and -rich enamels; SHPO $Sr_6H_3(PO_4)_5 \cdot 2H_2O$.

Line No.	Enamel			Hydroxyapatite (calc.)	SHPO	Other Sr phosphates	Accepted deviation (±) in *d*
	Powdered		Intact fragment				
	Sr-poor	Sr-rich*	Sr-rich*				
1	8.19†		8.09	8.16		0	0.08
2		6.64†	6.48		6.44	1†	.08
3	5.27	5.21	5.20	5.26		1	.08
4		4.33	4.36		4.38	3	.03
5		4.09(200)‡	4.06	4.08		2	.03
	3.87(111)‡	3.90(111)‡					
6	3.41(002)‡	3.45(002)‡	3.44	3.44	3.42	7	.03
7			3.35		3.32	8	.03
8		3.19	3.20	3.17	3.19	9	.03
	3.09(210)‡						
9			2.98		2.98	7	.03
10			2.87		2.85	8	.03
11	2.71, 2.75		2.73	2.72		6	.03
12	2.62	2.60	2.60	2.63	2.63	7	.03
13	2.52		2.53	2.53		5	.03
14		2.50	2.50			4	.03
15			2.39	2.36	2.39	9	.03
16	2.27(310)‡	2.27(310)‡	2.26	2.26	2.26	12	.03
17		2.17	2.18	2.21	2.17	8	.03
	1.94(222)‡	1.95(222)‡					
	1.71(004)‡	1.73(004)‡					

* Lines 18 through 41 had *d*-values consistent with hydroxyapatite. † Outside the accepted deviation, but indicating the presence of a reflection in this region. ‡ Used to determine lattice constants; reflecting plane appears in parentheses.

number of other strontium phosphates (*12*) that have a reflection corresponding to a particular *d*-value calculated from the fragment of strontium-rich enamel.

The $Sr_6H_3(PO_4)_5 \cdot 2H_2O$ contains many *d*-values common to an apatite, in addition to reflections, corresponding to the unique lines of the pattern of the intact fragment, with *d*-values of 6.48, 4.36, 3.35, 2.98, and 2.87 Å (Table 1). These results strongly suggest that $Sr_6H_3(PO_4)_5 \cdot 2H_2O$ was present in the incisor enamel. This view is supported by precipitation studies (*13*) showing that $Sr_6H_3(PO_4)_5 \cdot 2H_2O$ immediately precedes the formation of strontium hydroxyapatite.

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Table 1. Estimated amounts (micrograms per milliliter of culture medium) of gibberellin from cultures of fungi. Responses were compared to a dosage response curve for GA_3 with the *dwarf-5 Zea mays* assay. Assay response is the average of the sums of the length of the first and second leaf sheaths ($N = 5$).

Strains	Assay response (mm)	GA_3 equivalents (μg/ml)
	Parental	
2794a	*74 ± 4	2.40
2900A	89 ± 8	0.05
	Progeny, ascus 1	
1	*81 ± 3	3.75
2	*73 ± 4	2.16
3	66 ± 6	0.01
4	79 ± 6	0.03
	Progeny, ascus 2	
1	*71 ± 4	1.56
2	*84 ± 4	4.50
3	48 ± 3	0.00
4	68 ± 5	0.01
	Progeny, ascus 3	
1	*67 ± 5	1.05
2	*71 ± 9	1.56
3	86 ± 5	0.05
4	83 ± 4	0.04
	Progeny, ascus 4	
1	*87 ± 6	5.40
2	*81 ± 5	3.75
3	66 ± 4	0.01
4	77 ± 1	0.03
Controls	49 ± 3	

* Extract diluted 100-fold before assay.

Gibberellin Production: Genetic Control in the Fungus Gibberella fujikuroi

Abstract. *The fungus* Gibberella fujikuroi *(Saw.) Wr. can be used in genetic studies of the production of gibberellins. A gene has been identified which controls a step in the biosynthetic pathway of gibberellin production. Apparently this step is early in the pathway for it affects the accumulation of all of the gibberellins produced by the fungus.*

The gibberellins are a class of naturally occurring compounds that regulate a variety of growth and developmental processes in plants (*1*). While there is no evidence to indicate that gibberellins affect the growth of fungi, they are accumulated in large numbers and amounts by the fungus *Gibberella fujikuroi* (Saw.) Wr. (imperfect stage, *Fusarium moniliforme* Sheldon) (*2*). This report will show that *Gibberella fujikuroi* can be used to investigate the genetic control of gibberellin production. These studies provide evidence for a gene that controls the amount and kinds of gibberellins produced by the fungus.

Genetic studies with *G. fujikuroi* have been difficult in the past because of the inability to consistently obtain the sexual stage in the laboratory. However, perithecial production was recently induced when strains of opposite mating type were grown on a *Citrus* stem medium (*3*, *4*).

Crosses were made by the simultaneous inoculation of this medium with strains of opposite mating type (*5*). Mature perithecia developed in 3 to 6 weeks. They were removed and ruptured in water, and the asci were placed on a medium of 2 percent agar. The ascospores from an individual ascus were separated with a micromanipulator and transferred individually to slants of potato-dextrose agar (*6*).

Preliminary studies were made to determine whether ascus development was normal, that is, whether the ascospores from one ascus represented all four meiotic products (a tetrad). The strains mated in this study varied with respect to pigments that diffused into the medium, pigmentation of mycelium, and texture of the mycelial pad. On the basis of these visual phenotypes, cultures derived from the eight ascospores of one ascus could always be grouped into four pairs. This was interpreted as indicating that all four meiotic products were recovered from each ascus. Since the spore pair derived from each meiotic product could be identified, it was necessary to test only one member of each pair. Fifty-eight tetrads were also tested for mating type (*5*); in each tetrad there was the expected 2 : 2 segregation for mating type. Cultures classified as being derived from the same meiotic product always had the same mating type. We concluded that meiosis and ascus development in this fungus follow a conventional pattern.

The amounts and kinds of gibberellins produced by a strain were determined from extracts of the medium on which the fungus was grown. Cultures were grown for 1 week in 50 ml of liquid potato-dextrose medium in 125-ml erlenmeyer flasks. The cultures were maintained at 23°C on a rotary shaker (180 rev/min). After the incubation period, each culture was centrifuged to remove the mycelium. The supernatant medium was then adjusted to *p*H 2.5 and extracted three times with one-half volumes of ethyl acetate. The ethyl acetate fractions were combined, air dried, and assayed. The total amount of gibberellin-like materials in these extracts was estimated by the *dwarf-5 Zea mays* bioassay (*4*, *7*). Individual gibberellins were separated from each other by several thin-layer chromatographic systems (*8*); their presence and position on the chromatograms were determined by bioassay of eluates from the chromatograms and by fluorescence on the chro-