these peaks may have arisen by chance.

The contribution of the ten peaks of Table 1 to the total variance of the data is about 10^{-2}. A large portion of the variance is attributable to relatively slow changes, the low frequency components which make up the red-noise trend. The residuum after subtraction of the red-noise is a near-white spectrum of which the peaks contribute 6 percent. None of the peaks represent a constant, strictly periodic process; they vary in amplitude and for some time spans any given peak may be undetectable.

Although the peaks are statistically significant and must represent the effects of deterministic processes, either endogenous or exogenous, on the insulin requirements of this diabetic, this analysis cannot shed any real light on the nature of these processes. Periodicities of apparently endogenous origin in 17-ketosteroid excretion of a male at 6.9 to 7.0, 17 to 21, and 29 to 31 days have been observed by Halberg *et al.* (*12*). However, the notable absence of a peak at a 7-day period provides strong evidence against a role for social mechanisms. The $1/f$ trend and the lunar-related peaks both raise the possibility that weather-related phenomena are responsible.

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Calcium Oxalate Crystals in the Aragonite-Producing Green Alga Penicillus and Related Genera

Abstract. *Calcium oxalate crystals occur in the marine green algae* Penicillus, Rhipocephalus, *and* Udotea, *known as producers of sedimentary aragonite needles. In contrast to the externally deposited aragonite crystals which are generally* < 15 *micrometers long, the oxalate crystals are larger (up to 150 micrometers) and are located in the vacuolar system of the plant. No calcium oxalate was found in the related but noncalcifying genera* Avrainvillea *and* Cladocephalus.

Species of the green algal genus *Penicillus* and of the related genera *Rhipocephalus* and *Udotea* (Codiales) are significant producers of fine aragonite needles in marine sediments (*1, 2*). *Penicillus* is a major contributor of aragonite lime mud in the Florida Bay and near shore parts of the Florida reef flat, while *Rhipocephalus* and *Udotea* are more abundant in the reef tract and the outer margin of Florida Bay (*2*). The algae deposit aragonite as an external lime sheath on the surface of the cell wall and, upon death of the organism, the sheath disintegrates into individual crystals. The aragonite crystals have been described as needles < 15 μm long (*2, 3*); small prisms approximately 0.5 μm long and serrated crystals about 1.0 μm long also occur (*4*).

We found that, in addition to the externally deposited aragonite, intracellular crystals are also present, often in significant quantities. These are calcium oxalate needles up to about 150 μm long and are produced in the vacuolar system of the organism.

In the light microscope the crystals appear as single acicular structures (Fig. 1, b to i). The internal structure of a plant with intracellular crystals is shown in a living, young uncalcified capitular filament of *Penicillus capitatus* (Fig. 1b). The cytoplasm, containing numerous green plastids, appears as a thin, continuous parietal layer. The crystals are randomly oriented in the central vacuolar system (*5*). In the transmission electron microscope (Fig. 1j) the crystals appear to be encased in a chamberlike structure within the granular vacuolar material. Their appearance is similar to the electron microscopic image of calcium oxalate crystals in higher plants (*6, 7*).

For x-ray diffraction analysis the intracellular crystals were isolated from specimens of *P. dumetosus*, collected in the Florida Bay, by dissolution of the aragonite sheath in warm 65 percent acetic acid and subsequent digestion of the organic matter in 5.25 percent commercial sodium hypochlorite solution at 70°C. The acetone-washed and dried sample was mounted in a Lindemann glass capillary and photographed with Ni-filtered CuKα radiation in a Debye-Scherrer powder camera of 57.3 mm radius (Fig. 1a).

Visually estimated approximate intensities and observed *d*-spacings for our sample are listed in Table 1. There

Table 1. Diffraction pattern of intracellular crystals in *Penicillus dumetosus* and of $CaC_2O_4 \cdot H_2O$ form B from ASTM powder diffraction file 14-770.

P. dumetosus		$CaC_2O_4 \cdot H_2O$	
d-Spacing (Å)	Intensity (I/I_0)	*d*-Spacing (Å)	Intensity (I/I_0)
8.6	10		
6.2	80	6.13	80
4.4	40	4.34	40
3.9	15	3.84	40
3.67	15		
3.35	5		
3.08	18	3.07	40
2.77	100	2.75	100
2.41	30	2.40	60
2.34	10		
2.24	40	2.22	60
2.20	3		
2.12	20	2.10	40
2.02	10	1.99	10
1.95	20	1.94	20
1.89	20	1.89	60
1.83	20	1.82	40
1.78	4		
1.74	20	1.73	40
1.69	10	1.69	10
1.65	1		
1.62	2		
1.58	3		
1.56	10	1.56	10
1.50	10		
1.48	5	1.48	40
1.43	3		
1.42	5	1.42	20
1.385	15	1.38	40
1.315	10	1.32	20
1.268	1		
1.235	1	1.23	10
1.190	10	1.19	40
1.170	4	1.17	10
1.150	10	1.15	40
1.130	2		
1.115	1		
1.092	5	1.09	10
1.060	10	1.06	20
1.035	2		
1.014	2		
0.988	2		
0.951	1		
0.930	3		
0.845*	3		
0.788	2		

* Diffuse

exist several different forms of hydrated calcium oxalate. The diffraction pattern of our sample corresponds to that of one of the tetragonal monohydrates reported in the powder diffraction file of the American Society for Testing and Materials. Intensities and *d*-spacings from this source are compiled for comparison in Table 1. The minor discrepancies between the two patterns is not surprising in view of the variation in diffraction patterns of calcium oxalate monohydrate from different sources noted by Arnott and Pautard (*6*) and the possibility of slight contamination of our sample by unidentified material. We conclude that our sample is a form of calcium oxalate monohydrate.

We examined all American species of the genus *Penicillus* [*P. dumetosus* (Lamour.) Blainv., *P. capitatus* Lamarck, *P. pyriformis* A. et E. S. Gepp, *P lamouroxii* Dec.], both species of the genus *Rhipocephalus* [*R. oblongus* (Dec.) Kütz. and *R. phoenix* (Ellis et Sol.) Kütz.] as well as *Udotea conglutinata* (Ellis et Sol.) Lamour. and found calcium oxalate crystals in all (Fig. 1, b to i). Yet, capitular filaments with few or no intracellular crystals are not uncommon, as may be seen in the left branching in Fig. 1g. It may also be noted that Gepp's classical monograph of the Codiales (*8*) shows in plate I, his figure 12c, acicular crystals similar to those in our photographs, though unaccompanied by description, in *Udotea spinulosa* Howe.

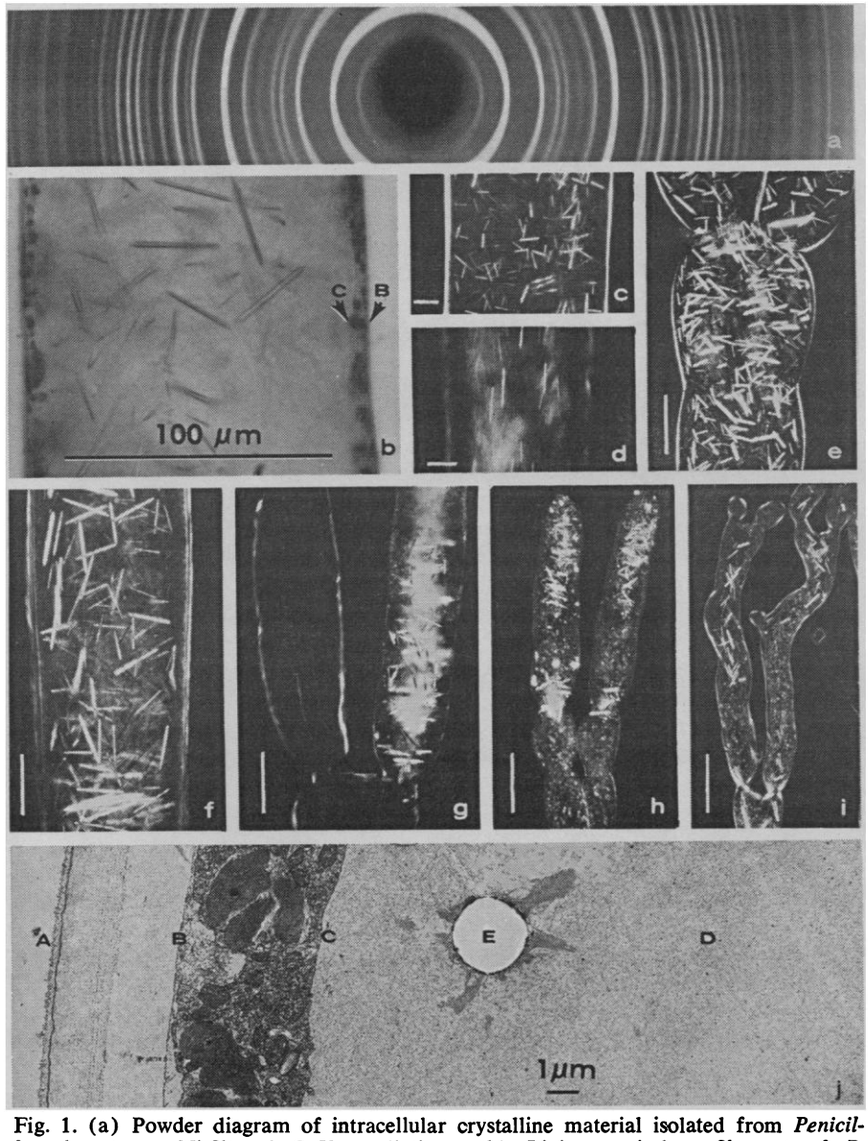

Fig. 1. (a) Powder diagram of intracellular crystalline material isolated from *Penicillus dumetosus*; Ni-filtered CuKα radiation. (b) Living capitulum filament of *P. capitatus* in the Leitz-Nomarski interference contrast microscope showing acicular crystals in the central vacuome. The peripheral cytoplasm layer with discoid plastids appears in dark contrast through the use of a Kodak Wratten 48 filter. (c to i) Intracellular acicular crystals in decalcified capitulum filaments of some Codiales in the polarizing microscope. (c) *Penicillus dumetosus*. (d) *P. lamourouxii*. (e) *P. capitatus*. (f) *P. pyriformis*. (g) *Rhipocephalus oblongus*. (h) *R. phoenix*. (i) *Udotea conglutinata*. (j) Cross section of a capitulum filament of *P. capitatus* in the transmission electron microscope, fixed in glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate (in electron microscope sections the mineral substance of the calcium oxalate crystals is, as a rule, removed during preparation). *A* to *B*, cell wall; *B* to *C*, cytoplasm with discoid plastids; *D*, granular vacuolar substance; *E*, calcium oxalate crystal chamber.

The genera *Avrainvillea* and *Cladocephalus* are morphologically similar to *Penicillus, Rhipocephalus*, and *Udotea*, but they are not calcifying. We did not find calcium oxalate crystals in either *A. nigricans* Dec., *A. longicaulis* (Kütz.) Murr. et Boodle, *A. levis* Howe, or *C. luteofuscus* (Crouan) Börg.

Intracellular calcium oxalate monohydrate is common in higher plants (*6*), although its presence in lower plants has only been occasionally reported [for example, in the Australian green alga *Apjohnia laetevirens* Harvey from the related order Siphononcladales (*9*)].

Our findings seem to be at variance with the results of Perkins *et al.* (*4*) who recently described intracellularly formed aragonite crystals in *P. dumetosus*. The size, morphology, and location of the crystals appear to be very similar to our calcium oxalate crystals. Thus, one has to assume either that morphologically identical intracellular crystals of two chemically different substances are present in the same organism or that the electron and x-ray diffraction samples of Perkins *et al.* were contaminated by aragonite from the external sheath of the plant. The observation of Perkins *et al.* that the intracellular crystals withstand decalcification seems to point toward the second possibility. A further difference exists in that Perkins *et al.* found intracellular crystals only in *P. dumetosus* (and by implication, not in *P. capitatus*, in *Udotea*, or in *Rhipocephalus*, which they also investigated) and suggest that the presence of crystals may be of taxonomic value in identifying *P. dumetosus*. This, according to our observations reported above, does not seem to be the case.

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5. *Penicillus* and the other allied genera dealt with in this report are coenocytic organisms; that is, they are multinucleate but without cross walls, and they are not divided into individual cells. The plants are composed of the branchings of a single filament interwoven into a complex and morphologically highly differentiated thallus which may attain the size of several decimeters. Because of the lack of cross walls the vacuome of a plant forms a continuous system.
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Fertility Impairment in Mice on a Low Fluoride Intake

Abstract. *Female mice maintained on a low fluoride diet over two generations showed a progressive decline in litter production. Mice receiving the same diet supplemented with fluoride reproduced normally and at consistent intervals. Addition of fluoride to the intake of females with demonstrated impaired fertility restored their reproductive capacity.*

Satisfactory evidence of a deficiency state with respect to fluorine has not been demonstrated despite several investigations with this purpose (*1*). We report here a progressive decline in fertility and a delayed onset of sexual maturity in female mice maintained on a low fluoride intake.

Weanling female albino mice were divided randomly into two groups and were given either deionized water (58 animals) or deionized water containing 50 parts per million of fluoride as sodium fluoride (55 animals). Both groups received a low fluoride diet (*2*) containing 0.1 to 0.3 ppm of fluoride. Commercial rodent food contains 30 to 80 ppm of fluoride. The mice were first mated at 8 weeks of age and were maintained in groups of four earmarked females and one male per cage. The males were housed constantly with the females for 25 weeks. To confine the dietary effects to the females, the male mice were fed laboratory chow prior to breeding and were transferred every 6 weeks between the female animals that were on a low fluoride diet and those on a high fluoride diet. Litter production was observed over the 25-week period to a maximum of four litters. Each litter was reduced to six pups and all pups were removed at 5 days of age to promote a rapid breeding rate. The age at which each mouse gave birth to its first and subsequent litters and the number of pups per litter were recorded. Body weights of 100 newborn pups and 50 5-day-old pups from each fluoride group were obtained.

Litter production was also assessed in second generation females taken mainly (*3*) from the fourth litters of the first-generation mice. These mice were maintained on the same fluoride intakes as their mothers and an experimental plan identical to that for the first generation was followed. The second-generation group which was on a low fluoride diet contained 38 females and the group receiving a high fluoride diet contained 44 animals.

Mice in both generations of both fluoride groups weighed approximately 13 g at weaning and 25 g at 8 weeks, when they were first mated. The mean age at which mice gave birth to their first litter was not significantly different from 13 weeks for both generations of the high fluoride group and for the first generation of the low fluoride group. However, a highly significant ($P < .005$) delay in the birth of the first litter occurred in the second-generation animals on the low fluoride intake—16.0 ± 0.87 (standard error of the mean) weeks versus 13.0 ± 0.52 (S.E.M.) weeks for the high fluoride second-generation animals.

Fertility was essentially complete in both generations of mice in the high fluoride group (Table 1); 96 percent of the first-generation and 90 percent of the second-generation mice given the high fluoride intake produced four litters within the experimental period. Neither of these values is significantly less than 100 percent. However, mice in the low fluoride group showed a progressive impairment in reproductive capacity. Thus, while all animals of the first generation produced one litter, progressively fewer mice gave birth to additional litters and less than 50 percent produced four litters. The decrease in reproduction was more severe in the second-generation mice on the low fluoride intake and a progressive decline in litter production was again apparent. Almost 20 percent of animals in this group failed to produce even one litter and more than 50 percent failed to produce four litters. The differences in litter production between high and low fluoride groups were significant for the third and fourth litters of the first generation and for all four litters of

Table 1. Influence of maternal fluoride intake on litter production over two generations. The percentage of mice producing a given litter in the 25-week experimental period and, in parentheses, the number of mice at risk, is given for each litter. Significance level of differences between high and low fluoride groups was determined by comparison of binomial distributions (*8*). Abbreviation: n.s., not significant.

Litter	Percentage of mice producing litter		Significance levels
	High fluoride diet	Low fluoride diet	
	First generation		
First	98.1 (55)	100.0 (58)	n.s.
Second	98.1 (53)	91.4 (58)	n.s.
Third	96.0 (51)	72.7 (55)	$P < .001$
Fourth	96.0 (51)	48.1 (54)	$P < .001$
	Second generation		
First	97.7 (44)	81.5 (38)	$P < .05$
Second	95.2 (42)	66.7 (36)	$P < .001$
Third	95.1 (41)	54.2 (35)	$P < .001$
Fourth	90.0 (40)	45.7 (35)	$P < .001$

Table 2. Influence of fluoride on recovery of fertility. The table lists the number of mice at risk in parentheses and the percentage of mice producing a given litter in 20 weeks following transfer to a high fluoride intake or retention on the low fluoride intake. The 5-week transfer group represents those mice transferred to the recovery study after 5 weeks of breeding on a low fluoride intake without producing a litter. The 15-week transfer group was placed in the recovery study after producing only one or two litters in 15 weeks of breeding while on the inadequate diet.

Litter	Percentage of mice producing litter	
	High fluoride diet	Low fluoride diet
	5-week transfer	
First	100.0 (15)	78.6 (14)
Second	100.0 (15)	78.6 (14)
Third	93.0 (14)	61.5 (13)
Fourth	85.8 (14)	38.5 (13)
	15-week transfer	
First	100.0 (12)	91.0 (11)
Second	100.0 (12)	81.8 (11)
Third	100.0 (11)	63.6 (11)
Fourth	91.0 (11)	54.5 (11)