served in Bodian-PAS (periodic acid–Schiff) stained sections. The ALS case without dementia originated from the ALS Center of St. Vincent's Hospital, New York City, and was supplied by Dr. Donnenfeld.

5. *Sheep and squirrel monkeys*. Normal and natural scrapie sheep brains were from the NINCDS collection of frozen brains. Squirrel monkey brains also originated from the NINCDS collection. Squirrel monkeys were inoculated with (i) tissue from a human case of CJD; (ii) blind passage tissue from a squirrel monkey inoculated with tissue from human AD; (iii) tissue culture explants of two human kuru cases; or (iv) a multiple passaged isolate of scrapie (NIH strain C506). In addition, a multiply passaged chimpanzee kuru isolate was transmitted by feeding to a squirrel monkey. All squirrel monkeys inoculated with unconventional agents were clinically affected when killed.
6. *Scrapie mice*. Female C57B1/6J mice, 8 weeks old, were inoculated intracerebrally with 0.03 ml of a 1 percent mouse brain homogenate from either normal or scrapie strain 139A clinically affected C57B1/6J mice. At the time of clinical disease (4 months) in the scrapie mice, all were killed by cervical dislocation. The brains were removed and frozen at −70°C until processed for SAF. Strain IM/DK mice were inoculated intracerebrally at 8 weeks of age with 0.03 ml of a 1 percent brain homogenate from a postmortem specimen from the brain of an AD patient. Two mice were killed, one at 14 months and the other at 18 months after inoculation. There were no clinical signs, and the routine histology was normal.
7. *Hamsters*. Outbred weanling female golden Syrian hamsters (Charles River–Lakeview Hamstery) received 0.05 ml of either a 10 percent suspension of normal brain or a 10 percent suspension of agent (strain 263K)–affected brain. During the terminal stage of the disease (2 months after inoculation), infected and control animals were killed by CO_2 asphyxiation. The brains were immediately removed and frozen in dry ice.
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27. Since our first publication of the SAS structure in 1981 (*1*), SAF has been isolated in three additional laboratories (*2, 26*, this report). More recently S. Prusiner *et al.* have also reported [*Cell* **35**, 349 (1983)] a rodlike structure isolated from scrapie-infected hamster brains by a method which should produce SAF. These rods have the larger diameter (25 nm) twists and featureless appearance expected when SAF are visualized by the method of rotary shadowing that they used. Negatively stained images of their preparation have the diameters of SAF (11 to 20 nm) and twists; several micrographs indicate that the rods are composed of two filaments. This suggests that the structure described is also SAF.

28. We thank Dr. Richard Kimberlin for providing scrapie strains 139A and 263K; Dr. Alan Dickinson for providing the IM/DK mice; Dr. Greg Atkins for providing the M9 mutant strain of Semliki Forest virus; B. R. Brooks for providing the neurotropic retrovirus, Lake Casitas strain; and our colleagues for their support and encouragement. Supported in part by AGO4220.

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Scoliosis in Chickens: Responsiveness of Severity and Incidence to Dietary Copper

Abstract. *The severity and incidence of spinal lesions were manipulated in a line of chickens susceptible to scoliosis by varying their dietary intake of copper. A decrease in expression of the lesion was related to increased intake of copper. The change in expression, however, appeared to be related only indirectly to the defects in collagen cross-linking, maturation, and deposition known to be associated with dietary copper deficiency. Thus, a dietary constituent in the range of normal intakes may act as an environmental factor in the expression of scoliosis.*

The etiology of the most common clinical varieties of scoliosis has yet to be established. Although investigators have implicated defects in muscular growth, neural development, and connective tissue proteins (*1*), the specific factors that underlie expression of the lesion are unknown. We report here that manipulation of dietary copper alters the severity and incidence of scoliosis in a line of susceptible chickens derived from White Leghorns (*2*).

Many features of scoliosis in this animal are similar to those observed in humans. For example, the lateral spinal curvature is expressed in the thoracic region, the severity of the lesion is often greater in the homogametic sex, and the curves occur predominantly before sexual maturation (*2, 3*). Moreover, there is increased solubility of skin collagen, elevated urinary hydroxyproline, and altered distribution or properties of collagen in vertebral disks (*1–3*).

We investigated the relation of dietary copper to scoliosis because copper has been established as a cofactor in collagen maturation (*4*). Increased intake of cop-

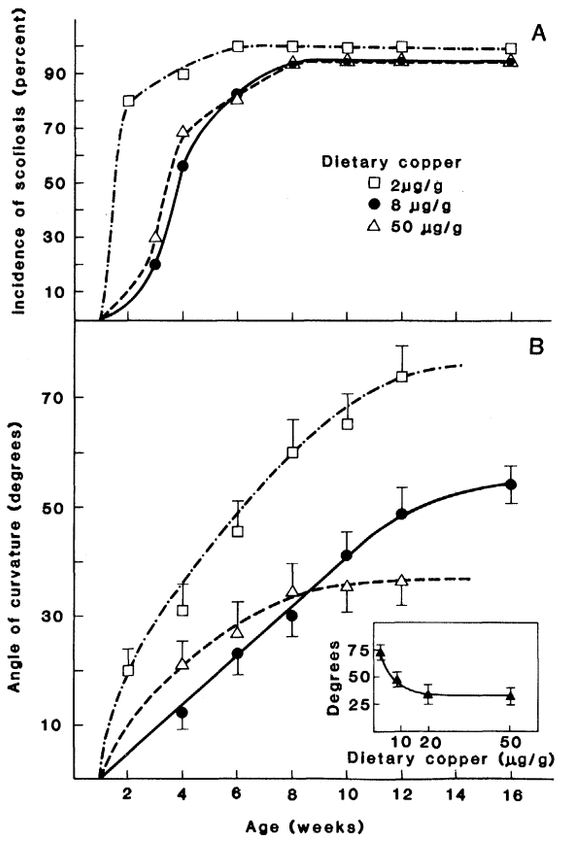

Fig. 1. Effect of dietary copper on the incidence (A) and severity (B) of scoliosis in susceptible chickens. Scoliosis was defined as any lateral curvature in excess of 10° (*9*). Each group contained at least 14 birds (equal numbers of males and females). At 12 weeks the expression of scoliosis exceeded 90 percent in both male and female chicks fed a conventional starter ration containing 6 to 10 μg of copper per gram. The average abnormal curvature was 44 ± 5 degrees in females and 50 ± 4 degrees in males (the homogametic sex in the chicken). The inset in (B) summarizes data for angle of curvature in the various groups at week 12. Values are means ± standard deviations.

Table 1. Indices of collagen maturation: response to dietary copper in normal and scoliotic chicks 3 weeks of age. Values (means ± standard errors) with different superscripts are significantly different from each other at $P < 0.05$ (analysis of variance and Duncan's multiple range test).

Phenotype	Dietary copper (μg/g)	Dietary manganese (μg/g)	n*	Bone copper (μg/g)	Body weight (g)	Lysyl-oxidase activity†	Collagen cross-linking (dpm/μg hydroxyproline)		Severity of lateral curvature (deg)	Incidence (%)
							HLNL	DHLNL		
Scoliotic	2	50	8	1.86 ± 0.12[a]	110 ± 6[a]	2730 ± 110[a]	5720 ± 261[a]	5981 ± 237[a]	35 ± 4[c]	100[c]
Scoliotic	6	50	5	1.71 ± 0.09[a]	121 ± 6[a]	4040 ± 410[c]	4401 ± 292[a]	7878 ± 337[b]	4 ± 4[b]	20[b]
Scoliotic	20	50	6	1.67 ± 0.14[a]	134 ± 9[a]	4200 ± 430[c]	4932 ± 189[a]	5320 ± 381[a]	7 ± 4[b]	50[b]
Normal	6	50	8	2.18 ± 0.14[b]	159 ± 17[b]	3050 ± 120[b]	6601 ± 409[b]	9336 ± 531[c]	0[a]	0[a]

*Number of observations. †Tritium released from the oxidative deamination of lysyl residues in an elastin- and collagen-rich substrate (*4*). Data are expressed as disintegrations per minute per gram of fresh bone per hour.

per increases lysyloxidase activity, the level of which is a major determinant in collagen cross-linking and maturation. Severe copper deficiency (dietary levels less than 1 μg/g) has resulted in scoliotic lesions in previous experiments with young normal chicks (*5*). Similarly, β-aminoproprionitrile, a lysyloxidase inhibitor, has been used to induce scoliotic lesions (*6*).

Newly hatched chicks of the scoliotic genotype were fed semipurified diets containing 2 to 50 μg of copper per gram (*7*). The lowest level of copper (2 μg/g) corresponds to a level compatible with at least marginal growth and normal connective tissue development in White Leghorn chicks (*4*). Onset and severity of scoliosis (*8, 9*) were then estimated, as were several other copper-related indices [vertebral bone copper (*10*), vertebral bone lysyloxidase activity (*4*), and selected cross-linking amino acids in vertebral bone collagen (*11*)].

The content of copper in the diet significantly affected the onset and severity of scoliosis (Fig. 1). Abnormal spinal curvature was usually observed around 3 to 4 weeks of age in chicks receiving copper at 6 to 10 μg/g; however, abnormalities as severe as that shown in Fig. 2 were sometimes observed as early as 2 weeks in chicks receiving 2 μg/g.

Dietary copper may regulate lysyloxidase activity and subsequent collagen cross-linking reactions, influencing the structure or integrity of vertebrae. Lysyloxidase activity in scoliotic chicks was elevated at the higher levels of dietary copper, although vertebral bone copper was not affected by the changes in dietary copper (Table 1). Levels of lysyloxidase activity were within normal limits, but the amounts of reducible cross-links in vertebral bone were lower in scoliotic chicks than in normal White Leghorn chicks at all levels of dietary copper. These results suggest that if collagen cross-linking is a factor in scoliosis, the process is more complex than may be attributed simply to dietary copper deprivation and decreased lysyloxidase activity. For example, the observed differences in collagen cross-linking between normal and scoliotic birds may reflect differences in the maturation of collagen fibers. An increase in the severity of the lesions was observed up to 12 weeks of age, even though the incidence varied little among treatment groups after week 8. Development of the disorder in birds receiving dietary copper at 50 μg/g seemed to be curtailed by week 8 (Fig. 1). Birds receiving 20 μg of copper per gram (Table 1) had a pattern of spinal development similar to that of birds receiving 50 ppm copper, and no increase in the angle of curvature was observed after week 8.

Fig. 2. Representative scoliotic spine from a 12-week-old male chicken fed a standard commercial starter ration (6 to 10 μg of copper per gram).

Dietary copper intakes vary widely in human populations. Several studies have shown that intakes are often below the suggested intake of 2 mg/day (*12*). The incidence of scoliosis in man varies from 0.4 to 10 percent, depending on the criteria used to evaluate severity (*1–3*). Thus the identification of copper as an environmental factor with the potential of influencing the expression of scoliosis is important. At the very least, our observations suggest that diet plays a role in the etiology of the disorder.

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7. Copper was added as cupric sulfate to a diet based on spray-dried skim milk [K. Buckingham *et al.*, *Proc. Soc. Exp. Biol. Med.* **166**, 310 (1981)]. The chickens were housed in stainless steel cages and given unlimited feed and deionized water from the day of hatching. In separate experiments, day-old chicks were given feed containing either 50 to 1000 μg of manganese per gram and 8 μg of copper per gram or 50 μg of manganese per gram and 25 or 250 μg of copper per gram. The rationale for examining the effect of manganese is related in part to the role of manganese as a cofactor for glycosyl transfer-

ases [L. S. Hurley, *Physiol. Rev.* **61**, 249 (1981); R. M. Leach, A. M. Muenster, E. M. Wein, *Arch. Biochem. Biophys.* **133**, 22 (1969).] Within these ranges of manganese and copper intakes, no differences in the severity or onset of scoliosis were observed. Results were identical to those shown in Fig. 1 for chicks (3 to 12 weeks old) given copper at 8 μg/g.

8. Birds were sedated and examined after anterior and posterior roentgenography of the spine (*1*, *11*).
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10. The fourth thoracic vertebra was used in the analysis of bone copper. After being cleansed of adhering tissue, the vertebra was dissolved in 70 percent nitric acid for copper analysis by atomic absorption spectrophotometry.
11. A midsection of bone was taken for estimation of the reducible cross-linking amino acids hydroxylysinonorleucine (HLNL) and dihydroxylysinonorleucine (DHLNL). The samples (50 mg) were demineralized in 0.2*M* EDTA and dialyzed. The residue was collected by centrifugation (10000*g* for 60 minutes) and lyophilized. One-milligram portions were then reduced with [^{3}H]$NaBH_3CN$ (96 mCi/mg, New England Nuclear) as described by S. P. Robins and A. J. Bailey [*Biochem. J.* **163**, 339 (1977)]. After hydrolysis, HLNL and DHLNL were identified and separated with the chromatographic system described by R. Stack *et al.* [*Appl. Environ. Microbiol.* **46**, 539 (1983)] and hydroxyproline was assayed as described by J. R. Woessner [*Arch. Biochem. Biophys.* **93**, 440 (1961)].
12. On a dry weight basis, human diets in the United States typically contain 3 to 5 μg of copper per gram, or an amount approaching the lower limit of the copper requirements of most animals [K. E. Mason, *J. Nutr.* **109**, 1979 (1979); L. Klevay, *Biol. Trace Elem. Res.* **5**, 245 (1981)].
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Pheromonal Control of Metamorphosis in the Pacific Sand Dollar, *Dendraster excentricus*

Abstract. *Competent larvae are induced to undergo metamorphosis by sand from a sand dollar bed or an aqueous extract of the sand. Gel permeation chromatography and high-performance liquid chromatography of the extract yielded a 980-dalton peptide that will induce metamorphosis between 10^{-6} and 10^{-5} molar. Extracts of whole adults and gonads were also able to induce metamorphosis, and adults can condition substrates to induce metamorphosis. Therefore, the initiation of metamorphosis in* Dendraster excentricus *is controlled by a pheromone released by adult sand dollars.*

Larvae of many benthic marine invertebrates settle and metamorphose after a prolonged planktonic phase. In numerous species the larvae have been shown to metamorphose preferentially in habitats that are suitable for the juvenile or the adult (*1*). Larvae apparently develop in the plankton until they are competent to metamorphose and remain so until they encounter cues associated with preferred benthic habitats. Cues have been shown in several species to be physical or chemical factors that initiate the developmental sequences of metamorphosis (*2*). I now report that larvae of the echinoid *Dendraster excentricus* are rapidly induced to metamorphose by a chemical secreted into sand by adult sand dollars.

Larvae grown in laboratory culture are competent to metamorphose after 4 to 6 weeks (*3*). When exposed to sand from a sand dollar bed, 82.5 ± 9.6 percent (mean ± standard deviation; four trials) metamorphosed within 24 hours; when exposed to sand collected from a similar, adjacent area without sand dollars, 2.5 ± 5.0 percent (four trials) metamorphosed. Competent larvae not exposed to sand but maintained under culture conditions have been kept competent for over 5 months with less than 5 percent metamorphosing spontaneously. Lyophilized, aqueous extracts of sand from a sand dollar bed will also rapidly induce metamorphosis. Of larvae treated with 5 mg of extract per milliliter, 92.5 ± 9.5 percent (four trials) metamorphosed within 1 hour (*4*). The same concentration of an extract of sand from outside the sand dollar bed induced 5.0 ± 5.7 percent (four trials) to metamorphose. These results indicate that an extractable component of the sand from sand dollar beds will induce metamorphosis.

Gel permeation chromatographs of extracts indicated that the activity eluted in a single peak (Fig. 1A). When compared with the standards (tryptophan and vitamin B_{12}), the active peak had a molecular size of 980 ± 110 daltons. By reversed-phase high-performance liquid chromatography (HPLC), the active peak from gel permeation chromatographs was shown to contain several components (Fig. 1B); however, activity was only detected in a single peak. Spectrophotometric scans of active fractions showed absorption maxima at 206 and 266 nm. Active fractions also reacted positively with the Lowry (biuret/folin–phenol) method for protein determination (Fig. 1A). Activity in fractions purified by gel permeation was significantly reduced by treatments with insoluble protease E (16.7 ± 9.8 percent; three trials), trypsin (46.7 ± 11.5 percent; two trials), or heat (0; two trials) (*5*). These findings indicate that the active substance is probably a peptide (*6*).

The dose-response curve of fractions purified by gel permeation showed that

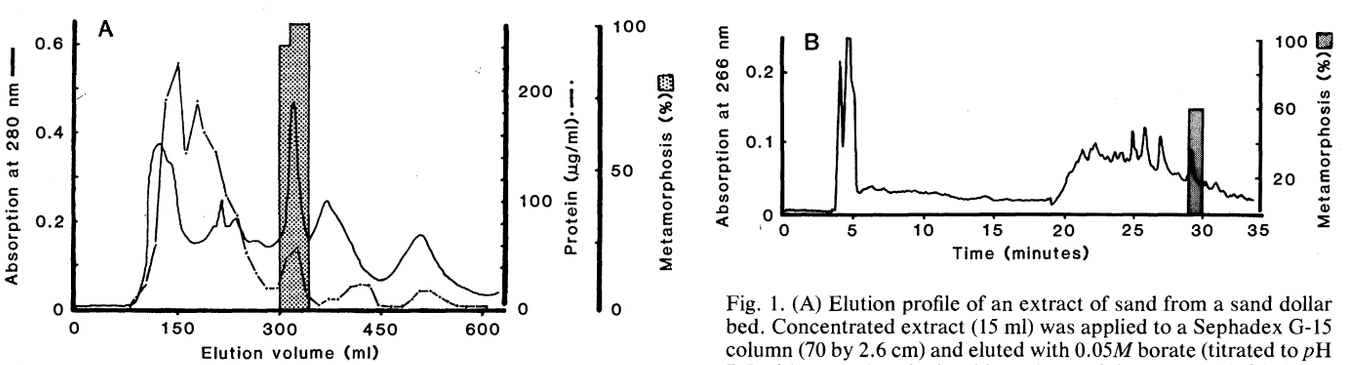

Fig. 1. (A) Elution profile of an extract of sand from a sand dollar bed. Concentrated extract (15 ml) was applied to a Sephadex G-15 column (70 by 2.6 cm) and eluted with 0.05*M* borate (titrated to *p*H 7.5 with potassium hydroxide and containing 0.03*M* NaCl). Flow rate was 1 ml per minute at 4°C, and 15-ml fractions were collected. Protein concentration of collected fractions was determined by the Lowry method (see text). For the bioassay, 0.5 ml of each fraction was mixed with 0.5 ml of twofold concentrated artificial seawater (ASW; Marine Biological Laboratories formula). Ten competent larvae were added, and the number metamorphosed was counted after 1 hour. (B) Separation by reversed-phase HPLC of the components of the active peak from a single gel permeation chromatography run. A Supelcocil LC-18 column (250 by 4.6 mm) with an LC-18 guard column was used with a Varian 5000 instrument and Vari-chrome detector. The sample was concentrated with a C18 Sep Pac (Waters), and a 0.8-ml sample was injected. The mobile phase was 10 percent acetonitrile (AN) and 0.25*M* formic acid [buffered to *p*H 6.5 with triethylamine (TEAF)] for the first 10 minutes. A 1 percent ascending gradient to 60 percent AN:TEAF was used to elute components. Flow rate was 1.0 ml per minute, and maximum pressure was 140 atmospheres. One-milliliter fractions were collected, concentrated with a vacuum centrifuge, and dissolved in 0.2 ml of ASW before being assayed for activity. The base line from a blank run, in which 0.8 ml of TEAF was injected, has been subtracted from the data presented.