Oral Proline Tolerance in Osteogenesis Imperfecta

Abstract. Studies were carried out to test the hypothesis that a disturbance in proline or hydroxyproline metabolism, or both, is associated with defective collagen formation in osteogenesis imperfecta, a generalized disorder of connective tissue. Oral tolerance for proline in affected patients was lower than in comparable controls.

In 1952 Follis (*1*) emphasized morphological similarities of connective tissue in osteogenesis imperfecta and scurvy. He demonstrated in both disorders formation of argyrophilic and metachromatic fibers which are characteristic of reticulum or immature collagen, and he proposed that a similar defect exists in the collagenous portion of bone matrix in both conditions. Robertson *et al.* (*2*) and Gross (*3*) have shown deficient formation of collagen in the scorbutic guinea pig, and Gould and Woessner (*4*) have postulated a failure of hydroxylation of proline as a basic defect in scurvy. These observations have assumed more important proportions when considered in the light of recent observations by electron microscopy of hydroxyapatite-crystal formation and growth which appear to begin in apposition to collagen fibers in osteoid matrix (*5*).

Proline, a nonessential amino acid, can be formed from glutamic acid and ornithine, and proline-C_{14} can be found in Kreb's cycle intermediates soon after introduction into the body (*6*). Stetten (*7*) has shown that proline can be readily hydroxylated to form hydroxyproline in the rat, and she has shown also that this reaction is not reversible. Orally fed hydroxyproline is incorporated not at all or to a limited degree into mature collagen. It has been pointed out by a number of observers that hydroxyproline is found primarily in collagen and in no other structural proteins, and it has been proposed that an activated proline, probably proline adenylate, is formed prior to its incorporation into the collagen polypeptide and its subsequent hydroxylation (*6*).

This study was undertaken to determine whether a rise in serum hydroxyproline could be detected after oral administration of proline in patients with osteogenesis imperfecta and in suitable controls. Lack of hydroxylation of proline or lack of incorporation of either proline or hydroxyproline into collagen might be expected to be reflected by abnormalities in the blood levels or urinary excretion of these amino acids. There is ample precedence for the belief that an enzymatic defect in the metabolism of a given substrate is reflected in abnormalities in the disappearance curve when the organism is subjected to a load of the substrate.

Serum levels of proline were determined for 8 hours after oral administration of proline (65 mg/kg of body weight) to seven children, ages 6 to 12 years, with osteogenesis imperfecta congenita (*1*) and tarda (*6*), and nine children, ages 6 to 12 years, who were normal (*5*) or had nonmetabolic orthopedic conditions (three cases of club feet and one case of mild cerebral palsy). All subjects had been on a regular diet and were fasted 12 hours overnight prior to and during the test. Proline was determined by a modification of the method of Troll and Lindsley (*8*). Hydroxyproline in the blood and urine was determined by a modification of the Wiss method (*9*).

Figure 1 demonstrates a difference in the averaged tolerance curve of the control group of children and the curves for affected children with osteogenesis imperfecta. A striking difference in the curves is noted especially in the first 2 hours of the tolerance test. Normal 95-percent confidence limits are shown on the curve of the control subjects for each blood specimen obtained. Three other patients with the congenita form of the disease have demonstrated low oral tolerance curves for proline. Patients with the tarda form of the disease approached normal values. Urinary clearance of proline is within normal limits during the test [0.2 to 0.5 ml/min (*10*)]. Preliminary results indicate that free hydroxyproline blood levels rise two to three times the fasting level (2 to 6 μg/ml) after oral administration of proline (65 mg/kg of body weight) to patients with osteogenesis imperfecta and control subjects.

It is apparent that no gross "diabetic-type" proline tolerance curve has been demonstrated in osteogenesis imperfecta similar to that observed with phenylalanine in phenylketonuria. Instead, lower maximum values were uniformly demonstrated in patients with this disease than in suitable controls. This finding could be explained by a gastrointestinal absorptive defect or more rapid incorporation of proline into metabolic pathways in patients with osteogenesis imperfecta. Urinary loss was excluded. It would appear that an absorptive defect for proline is not the primary lesion in osteogenesis imperfecta since this factor alone would fail to explain the findings present at birth in the more severely affected children.

In the study of osteoporotic bone conditions, the investigator is still confronted with many unanswered questions. If osteoporosis is related to deficient collagen synthesis or synthesis of an abnormal collagen, how does this affect calcification, crystal aggregation, and growth? Whether low oral tolerance

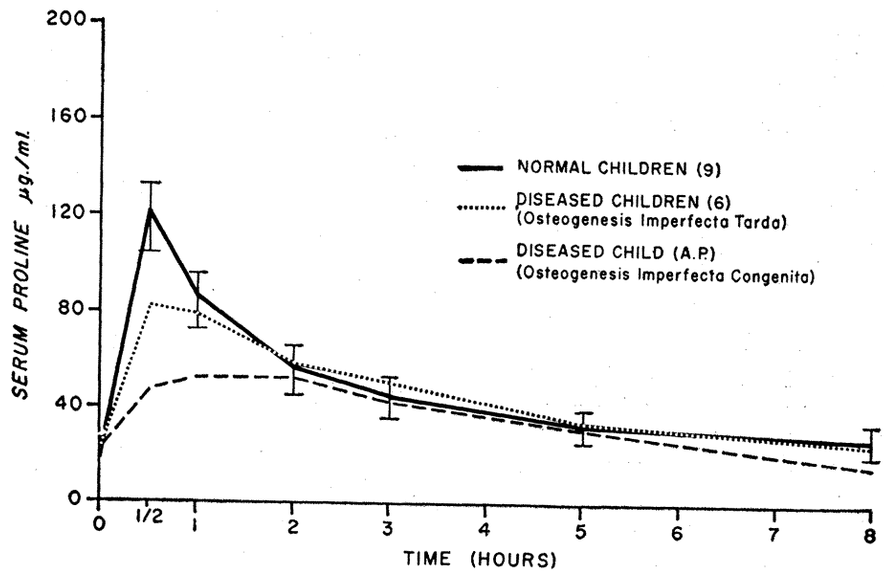

Fig. 1. Levels of proline in serum after oral administration of 65 mg of proline per kilogram of body weight.

for proline reflects a basic disorder in metabolism which significantly influences the formation of immature collagen in osteogenesis imperfecta cannot be answered from this study. A hydroxylating defect could not be demonstrated by the present techniques. It is noteworthy, however, that proline, one of the principal constituents of the polypeptide chain of collagen and also the precursor of hydroxyproline, should have a low tolerance curve when the amino acid is administered orally (*11*).

GEORGE K. SUMMER

Department of Pediatrics,
University of North Carolina
School of Medicine, Chapel Hill

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Longevity of Fusarium oxysporum in Soil Tube Culture

Abstract. In soil tube culture, representatives of three biologic forms of *Fusarium oxysporum* survived unchanged morphologically for 11 years or more. An isolate of the muskmelon wilt fungus remained viable after 17 years' storage in dry air at a temperature of from 3° to 4°C. The surviving unit was found to be the chlamydospore.

The traditional method of maintaining fungus cultures by making transfers from them at frequent intervals to artificial media is laborious and time-consuming and is not satisfactory for maintaining organisms unchanged for long periods of time. Other more or less dependable methods have come into use during the last 30 years, but some of them are applicable only to specific organisms.

Maintenance of fungus cultures in tubes of sterilized soil has found acceptance among some plant pathologists. Miller *et al.* (*1*) showed that when the muskmelon wilt fungus *Fusarium oxysporum* f. *melonis* (L. and C.) Snyder and Hansen and other fusaria are maintained on agar, the "wild type" is rapidly displaced by mutants. He reported success in maintenance of the "wild type" and retention of viability of the melon pathogen after it had been cultured for 15 months in soil tubes. Gordon (*2*) affirmed the advisability of using soil culture as a means of preserving *Fusarium* species. Atkinson (*3*) found that representatives of six of 32 genera of fungi were viable after 5 years in dried soil culture. Members of *F. oxysporum* were found in the surviving group.

The study reported here was specifically concerned with an investigation of the longevity and the means of survival in soil culture of isolates of *F. oxysporum* causing wilt of three different hosts.

From 1946 until 1953, stock cultures of *Fusarium oxysporum* f. *melonis* were prepared, and from 1948 until 1953, cultures of *F. oxysporum,* f. *lycopersici* and f. *niveum,* the causal agents of wilt of tomato and watermelon, respectively. The following procedure was used. Test tubes of 25-cm³ capacity were two-thirds filled with sandy soil containing 10 percent of muck. The mixture was saturated with water, and the tubes were plugged and autoclaved. The fungi were single-spored, after 3 days' growth, from platings of infected plant tissue on 2-percent potato dextrose agar. When monosporous cultures were 3 or 4 days old, a small weft of mycelium from the periphery of the colony was transferred to the soil tubes. The resultant cultures were allowed to incubate in the laboratory at room temperature in a diffuse diurnal light for from 2 to 4 weeks before they were transferred to a refrigerator for storage at from 3° to 4°C.

Recently, the viability of many of the stock cultures was determined by plating soil suspensions (0.01 to 0.04 g) on a modified Martin's peptone agar medium, as described by Snyder *et al.* (*4*). The results are given in Table 1.

Table 1 shows that the three biologic forms of the soil-borne, wilt-producing fungi showed varying capacity for survival. One isolate from muskmelon showed exceedingly high survival after 13 years, and another, appreciable survival after 17 years. A few cultures contained no viable units. Some cultures were found to be contaminated with bacteria. The presence of the latter, however, did not appear to be the reason for nonsurvival of the fungus, because several bacterial-contaminated cultures showed high viability of the pathogen after 13 years.

Microscopic examination of culture plates inoculated with soil suspensions revealed that the fungus was surviving as chlamydospores. These findings are in agreement with those of Warcup (*5*) and Nash *et al.* (*6*), who isolated other species of *Fusarium* directly from the soil and found the surviving unit to be a chlamydospore.

In general, chlamydospores are spherical, varying in diameter from 9 to 14 μ (average, 12.6 μ). The wall of the chlamydospore varies from smooth to slightly warty. Chlamydospores are found to contain from one to three oil globules. The chlamydospores occur singly and are usually firmly embedded in particles of organic matter, and often it is necessary to crush the particles before plating to determine the characteristics of the chlamydospore. On germination, chlamydospores have been observed to produce a single germ tube.

Growth of chlamydospores on various culture media showed that isolates of the three biologic forms of *Fusarium oxysporum* survived the long storage periods morphologically unchanged. Furthermore, comparative tests with fresh isolates showed that stored cultures of these three fungi survived without loss of pathogenicity.

Table 1. Survival of *Fusarium oxysporum* after storage in soil tubes at 3° to 4°C.

Isolate	Number of years in storage	Number of surviving units per gram of soil
F. oxysporum f. *melonis*		
Miller*	17	200
McKeen	15	1,200
Waters	13	3,300
Waters	13	134,000
Robinson	12½	7,400
Quick	12½	4,400
Wigle	12½	200
Setterington	12½	74,000
Murray	8	32,000
Larabee	8	300
F. oxysporum f. *lycopersici*		
Miller*	17	0
McKeen	12	95,600
Tecumseh	10	9,000
F. oxysporum f. *niveum*		
Waters	11	33,000
Tingen	11	22,000

*These cultures were prepared by J. J. Miller at the Harrow Laboratory in 1944.