Alkaline phosphatase activity in cultured skin fibroblasts from fibrodysplasia ossificans progressiva

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Summary. Alkaline phosphatase activity in four strains of cultured skin fibroblasts obtained from a patient with fibrodysplasia ossificans progressiva was at the low normal range. The enzyme activity in normal fibroblasts significantly increased at late confluency. It appears that the high levels of alkaline phosphatase activity reported in biopsies of lesions are not genetically determined but are secondary events of local tissue reaction.

Fibrodysplasia ossificans progressiva is a rare disease manifested by localized, often painful swellings, which gradually ossify and eventually lead to heterotopic bone formation. Microdactyly is an early manifestation of the disease, the great toe being the most frequently affected.

The vast majority of the cases appear to be sporadic. However, there are several reports of father to son or daughter transmission (Tünte, Becker, and von Knorre, 1967), as well as pairs of affected monozygous twins (Vastine, Vastine, and Arango, 1948; Easton, Conkling, and Daeschner, 1957). It seems that the disease is inherited as an autosomal dominant trait and most cases represent fresh mutations. An advanced paternal age of patients with fibrodysplasia ossificans progressiva has been noted by Tünte et al (1967).

The disease primarily affects the connective tissue and not the muscle fibres per se. The basic metabolic defect, however, is unknown. Wilkins, Regen, and Carpenter (1935) reported increased alkaline phosphatase activity in biopsies of lesions in the preossification stage, as well as in heterotopic bone and cartilage. The activity in the fibrous tissue was much higher than in the muscle. High enzyme activity was also found in biopsy specimens obtained from areas of muscle by Lins and Abath (1959) and Smith et al (1966). High alkaline phosphatase activity has been seen in osteoblasts and chondrocytes inside ectopic ossification sites (Stanescu, Dumitrescu, and Ionescu, 1967). In contrast, Letts (1968) found normal alkaline phosphatase activity by histochemical methods in non-ossified portions of a muscle biopsy. Cultured skin fibroblasts obtained from the skin and muscle of a patient were reported to have high levels of alkaline phosphatase activity (Herrmann et al, 1969).

We report the alkaline phosphatase activity in cultured skin fibroblasts obtained from a patient with typical fibrodysplasia ossificans progressiva.

Patients and methods

The patient, a female (AE220458), was born after a normal pregnancy. Her father was 37 years old when she was born. Both parents and her only sib, an older brother, were normal. The perinatal period and the first two years of life were uneventful. At the age of 2 she had acute episodes of arthralgias, followed by progressive stiffness of the involved joints. The elbows, the neck, and the entire spine were mainly affected. Gradually, painful localized swellings appeared in many areas over the neck, back, and extremities. By the age of 16 years there was immobilization of the trunk, shoulders, neck, right elbow, and mandible. The temporo-mandibular joint was virtually immobile; she fed by pushing food between spaces in her teeth. There were multiple nodules on the arms, legs, neck, and back. There was a firm mass in the left gastrocnemius region. The thumbs and first toes were short (Fig.).

Radiological examination showed soft tissue calcifications in the chest wall, extremities, submandibular area, and right masseter. There were exostoses of the mid and distal femurs. The proximal and distal phalanges of both first toes were short and fused. Fusions were
Results and discussion

The Table lists the alkaline phosphatase activity observed in cultured skin fibroblasts of the patient and the 10 control subjects. No increase in the enzyme activity was seen in 4 fibroblast strains that grew from the skin biopsy of the patient. In fact, the mean activity in the patient's fibroblasts was slightly lower than in the control subjects, though overlap between enzyme activities in the patient and normal donors was present. In three normal fibroblast cultures harvested at late confluency the mean activity was 23.46 nmole p-nitrophenol per mg of protein per hour (range 11.46 to 48.59). The activity in the same three cultures harvested at early confluency was 3.74 (range 2.45 to 5.77). This indicates that alkaline phosphatase activity is several times higher in fibroblasts at late confluency than in early confluency. Therefore, care should be taken to harvest the cells at the same growth phase when quantitative studies of the enzyme are performed. We cannot interpret the earlier reported increased alkaline phosphatase activity in cultured fibroblasts of a patient (Herrmann et al., 1969), since a non-quantitative histochemical technique was used and cultivating conditions were not mentioned.

![Image](http://jmg.bmj.com/)

**Fig.** The patient at the age of 16 years.

also present between the proximal and middle phalanges of the other toes except the second right toe. The blood chemistry, including calcium, phosphorus, and alkaline phosphatase was within normal limits.

A skin biopsy was obtained from a healthy area of the inner surface of the forearm of the patient and from 10 normal controls. Skin fibroblasts grown from the biopsy tissue were cultivated in RPMI 1640 medium supplemented with 20% fetal calf serum, 2 mmol L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells used for enzymatic assays were derived from cultures at the early confluent stage between the third and eighth subculture. Three cultures derived from normal subjects were also harvested at late confluency. Fibroblasts were always harvested 24 hours after the last feeding by exposing them for 2 minutes to 0.25% trypsin and subsequently to 0.02% sodium ethylenediamine tetra-acetic acid in isotonic saline for 20 minutes. Cells were washed three times with isotonic saline and lysed in water by seven cycles of rapid freezing and thawing. The lysates were centrifuged at 1800 × g for 5 minutes and the supernatant fractions were used for enzyme assays.

Alkaline phosphatase activity was determined using p-nitrophenyl-phosphate (18 mmol) in 0.1 mol carbonate-bicarbonate buffer, pH 10.5. The reaction mixture was incubated for 3 hours and the reaction was stopped with NaOH. Protein was measured by the method of Lowry et al. (1951). All enzyme determinations were carried out in duplicate.

**TABLE**

<table>
<thead>
<tr>
<th>Subject (No.)</th>
<th>Enzyme Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Patient (1)</td>
<td>2.59†</td>
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<tr>
<td>Control (10)</td>
<td>4.26 ± 1.14</td>
</tr>
</tbody>
</table>

* nmole p-nitrophenol per mg of protein per hour.  
† Mean of four fibroblast strains.

Since it has been shown that cultured skin fibroblasts usually express the genotype of the donor through successive generations in culture, they are preferable to biopsy specimens for the distinction between primary and secondary metabolic events. This is because the cultured cells grow in the laboratory under controlled conditions isolated from interactions with other tissue cells, abnormal local events, or various environmental factors secondary to the disease. The normal alkaline phosphatase activity found in cultured skin fibroblasts of the patient suggests that the high levels of enzyme activity observed in biopsy tissues are not genetically determined, but are secondary events associated with calcification and heterotopic bone formation.
Alkaline phosphatase in fibrodysplasia ossificans progressiva

REFERENCES


