week in a controlled environment of 16 hour day length; 11,000 lu/m^2; air temperature 20°C; growth solution temperature 12° to 15°C. Seedlings were subsequently grown under the same conditions in full strength solution, which was changed weekly. Attached roots of 2- to 4-week-old plants were placed in an aqueous solution of uranyl acetate (10^{-3} or $10^{-4}M$) for the required period. After thoroughly rinsing in distilled water, segments were excised from different parts of the root, fixed for 6 hours in 1.5 percent glutaraldehyde buffered to *p*H 7.2 to 7.3 in 0.05*M* sodium phosphate, washed for more than 20 hours in six changes of 0.15*M* buffer, fixed for 5 hours at 4°C in 1 percent osmium tetroxide buffered to *p*H 7.2 to 7.3 in 0.1*M* sodium phosphate, and washed in 0.1*M* buffer. Dehydration was through an acetone series via propylene oxide into epoxy resin [H. H. Mollenhauer, *Stain Technol.* **39**, 111 (1964)]. Sections were cut on an LKB III ultramicrotome, picked up on uncoated copper grids and examined in AEI EM6B or Hitachi HS8 electron microscopes.

2. H. Wheeler and P. Hanchey, *Science* **171**, 68 (1971).
3. Sections were examined from the following zones: root cap (0.5 mm); meristematic zone (> 0.5 mm); expansion zone (2 to 3 mm); endodermal walls without Casparian strip (< 5 mm); Casparian strip present, but no further thickening (> 7 mm); suberinized lamella and tertiary wall deposition taking place (> 8 cm). In the latter case, there is a long zone (≃ 20 cm) within which endodermal wall thickening is very asynchronous: thin-walled cells can be found adjacent to cells with extremely well-developed walls. A full account of the development of the endodermis in barley roots is in preparation.
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5. We thank the Science Research Council for supporting this work, and Dr. D. T. Clarkson for helpful discussions.

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Electrical Behavior of Cartilage during Loading

Abstract. *When cartilage is deformed, it becomes electrically polarized. At least two mechanisms seem to underlie this phenomenon, namely, a short-duration, high-amplitude, piezoelectric-like response and a longer-duration, lower-amplitude response secondary to streaming potentials. The polarity of articular cartilage during loading could hypothetically facilitate joint lubrication.*

Mechanically induced electrical polarization has been observed in a variety of biological systems (*1*). This polarization (charge separation) has been thought to occur mainly as a result of deformation of structural, long-chain, crystalline biopolymers, such as collagen, cellulose, protein-polysaccharides, and keratin. The precise mechanism behind this polarization is not yet clear, although "piezoelectric," pyroelectric, solid state, and electret properties have been identified in various tissues (*2, 3*). Furthermore, streaming potentials have been observed (*2*). Regardless of origin, mechanically and "hydraulically" induced electrical polarization possibly exert a major influence on the behavior of cells, ions, and charged macromolecules and on the organization of biowater (*2, 4*). In fact, growth and regeneration can be regulated electrically (*5*). This being the case, the present study was undertaken to determine the electromechanical properties of hydrated epiphyseal cartilage as an initial step in an attempt to alter its growth pattern electrically. In the process, other types of cartilage also were investigated. The investigation demonstrates that cartilage becomes electrically polarized when subjected to a deforming force and that the polarity of the polarization may have functional significance.

A total of 30 cube-shaped specimens were removed from the upper and lower femoral and tibial epiphyses of freshly killed term fetal calves, from 3-week- and 12-week-old calves, and from 10-week-old white New Zealand rabbits. The specimens included the epiphyseal plate, sandwiched between bone of the metaphysis and the ossification center (when present). Cubes of bovine epiphyses, measuring approximately 1 by 1 by 2 cm, were loaded so that the compressive force was normal to the epiphyseal plate. Compressive loads, at a rate of 10 cm/sec, were applied to the specimens with a Bytrex load cell, with an insulated tip, mounted on a pneumatically driven plunger. Electrical polarization in the specimens was detected by means of two cotton wick, Ag-AgCl electrodes, one wick contacting the epiphyseal plate, the other wick the metaphysis or ossification center. Specimens were held between electrically insulated surfaces and mounted in a Faraday cage in which the humidity and temperature were controlled. Details of the general procedure have been published previously (*6*). The amount of deformation (linear potentiometer), load, and electrical activity were recorded simultaneously with a type-R Dynograph and appropriate couplers (input impedance of the electrometer greater than 10^{10} ohms). For high-speed recordings, a Philbrick SPMD-100 operational amplifier (input impedance greater than 10^{10} ohms) was coupled to a Tektronix 564 oscilloscope.

In addition to the epiphyseal cartilages, 36 small cubes of fresh fetal and postfetal calf cartilage from the trachea, articular surfaces, menisci of the knee, and costal regions were tested. All samples were kept fully hydrated by storing in Ringer-Tyrode solution during shipment from the abattoir and during processing. Immediately prior to testing in the chamber, excess moisture was removed from the surface by blotting.

Epiphyseal samples were deformed in single steps through a range of 0.5 to 2 mm. The associated load, which initially reached values from 1 to 9 kg. decayed an average of 20 percent during the first 10 seconds and very slowly thereafter. For example, 60 seconds after the onset of a step deformation, 60 percent of the initial peak load was still present in the term calf specimens. As the specimens were deformed, electrical potentials, ranging from 0.5 to 2 mv, were recorded. The epiphyseal plate always was negative, relative to the surrounding bone of the metaphysis or ossification center. Electrical potentials decayed slowly, although at a rate faster than that generally observed for load decay. During the first 10 seconds, the initial peak potential decayed an average of 45 percent. The rate of electrical potential decay after the first 10-second period varied greatly from sample to sample, but rarely reached the baseline, even after periods in excess of 3 minutes. On release of the deforming load, a very small or no "reverse spike," indicative of a potential with a polarity opposite to that recorded on compression, was observed. This behavior differed greatly from that reported for bone (*6*). Generally, specimens with a very thick cartilaginous plate or a completely cartilaginous epiphysis (prior to the appearance of an ossification center) produced potentials of lowest amplitudes (0.5 to 1 mv). Furthermore, the decay rate of load during compression was greatest in these samples; frequently it had fallen 65 to 70 percent of the initial value at the end of the first 10 seconds.

After a complex sample, consisting of bone and cartilage, had been tested, metaphyseal and epiphyseal bone was dissected from the epiphyseal plate and each component was tested separately in compression. Electrode wicks were placed on adjacent faces of the cubes (90° from each other). Rarely were electrical polarization values of more than 100 to 200 μv recorded from the cancellous bone, while the polarization in the cartilage alone ranged from 0.5 to 1.5 mv for comparable ranges of deformation (0.5 to 2 mm). Changes in orientation of the epiphyseal plate sample, with regard to the direction of the compressive load, did not produce a significant change in voltage values.

All cartilages from the other areas produced electrical polarization on deformation, and the waveforms were similar to those already described for the epiphysis, that is, essentially uniphasic. At slow sweep speeds, an initial high potential value was followed by an immediate and rapid decay to a level approximately 30 to 60 percent of the initial peak amplitude (Fig. 1). This value was then maintained for very long periods of time (that is, minutes) with very little further decay.

At rapid sweep speeds, the onset of load and polarization occurred simultaneously, with peak values being reached within the first 2 msec. Peak voltage was followed by a very prompt decay (1 to 2 msec) to an intermediate value which then was maintained for a longer period of time (Fig. 2). There was one exception to this basic behavior. Costal cartilage generally gave rise to a distinct biphasic waveform with the "on spike" of compression having a polarity opposite to the "off spike" produced by release of the deforming load.

The lowest voltages were produced by tracheal cartilage (100 to 400 μv) and the highest by articular cartilage and meniscus (from 1 to 4 mv). Loading of the latter tissue normal to its dominant fiber axis produced much greater polarization for a given load than an equivalent load applied parallel to the long axis of the fibers. The joint surface of the articular cartilage always was positive with respect to cartilage near the tide mark or basal zone.

Extraction of the specimen with 0.05*M* NaOH or 0.05*M* HCl, or immersion in 3 percent NaCl for 48 hours, prior to testing, altered the electrical behavior significantly, but did not affect the mechanical properties to an appreciable extent. Each of these treatments abolished the long-term polarization which normally was sustained as long as the load was applied. The amplitude of the initial sharp "spike" associated with the onset of deformation was not altered significantly but the decay rate was markedly increased in these specimens (that is, long-term polarization disappeared). Replacement of the 3 percent NaCl by normal saline or Ringer-Tyrode solution for several minutes to several hours prior to test tended to reestablish the sustained longer-term polarization.

These experiments indicate that at least two types of charge separation occur in cartilage. One, the initial, high-amplitude, short-term "spike," probably arises from a "piezoelectric"-like phenomenon (mechanically induced, intra- or intermolecular charge separation or dipole reorientation). The second, a long-term, low-amplitude, portion of the waveform, conceivably arises from streaming potentials ("hydraulically" induced, extramolecular charge separation). Each type of behavior has been proposed, as a separate entity, previously. Shamos and Lavine's studies (*7*) of dry cartilage demonstrated a "piezoelectric"-like response to deformation but did not detect streaming. Maroudas (*8*), on the other hand, employed a method of study which demonstrated streaming potentials but which did not detect "piezoelectric"-like phenomena. The extraction and soaking procedures modified the streaming potential phase of the waveform but did not alter significantly the mechanical behavior. It is unlikely, therefore, that this phase of the waveform is dependent upon a slow stress relaxation with resultant intra- or intermolecular charge separation. The present study reemphasizes the need to consider the "total bioelectric response" to deformation as a sum of all the electrical events occurring in a tissue, whether additive or subtractive.

The significance of the polarity of the epiphyseal plate, vis-à-vis the metaphysis or the ossification center, is obscure at the moment, unless it is a reflection that, in general, regions of growth are characteristically electronegative. Conceivably, the cyclic deformation to which these growth structures are subjected by the activities of youth could be involved in maintaining, through electrical events, cell proliferation, nutrition, and function in the epiphyses. Certainly, the polarity of the epiphyseal plate should be given attention in any attempt to induce artificial currents in this structure for the purpose of altering its growth patterns. The significance of the polarity of articular cartilage, on the other hand, seems somewhat clearer. Joint lubrication during loading occurs largely as a result of the adherence of sodium hyaluronate to the articular surface. This biopolymer is a strong polyanion and would be expected to adhere more effectively to a positively charged surface than to one which was negatively charged. Since cartilage itself is fabricated to a large degree of protein-polysaccharides, which are negatively charged, it would seem appropriate to assume that Nature developed an electrostatically based method to facilitate cartilage lubrication at the moment of loading.

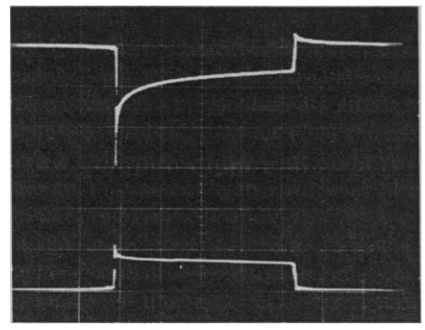

Fig. 1. Calf meniscus loaded perpendicular to dominant fiber axis. Tektronix oscilloscope; sweep speed, 0.5 sec/cm. Top trace, voltage; bottom trace, load. Note initial voltage spike with rapid decay and sustained potential until release of load. Load relaxation parallels voltage decay.

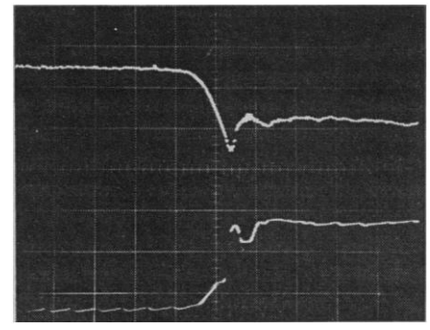

Fig. 2. Fetal calf condyle cartilage (articular surface). Sweep speed, 2 msec/cm. Top trace, voltage; bottom trace, load. Note parallelism between onset of loading and polarization. Initial voltage spike in this specimen decays approximately 40 percent in less than 1 msec.

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