

confined to a well-separated peak with a reproducible retention time, illustrated within dashed vertical lines in Fig. 1. Fifteen to thirty percent of the active material was recoverable with this trapping procedure.

In procedure No. 2 the first two liquid adsorption columns were used as previously, and the concentrated active eluates from the second column applied in two aliquots to a freshly packed gas chromatographic column. Trapping of the effluent stream was now carried out at -160°C in a bath of isopentane and liquid nitrogen. The two aliquots of attractant solution gave reproducible chromatograms of the form shown in Fig. 2A, the attractant peak being denoted by the dashed vertical lines. The longer retention time in Figs. 2A and 2B, as compared to Fig. 1, is attributable to the fact that a separately prepared batch of column packing was used.

The graph of Fig. 2A, adjusted to an amplifier gain of 1, shows that the amount of active substance was minute compared with the contaminants. The trapped active material from the two aliquots was combined and rerun in the same column at a gain of 30, as shown in Fig. 2B. This graph shows that the attractant was effectively separated in the first gas chromatogram and is now free from evident contamination. Seventy-five percent of the total peak area attributable to the attractant on the chromatograms of the two aliquots (see Fig. 2A) was observed on the chromatogram obtained from the combined eluates (see Fig. 2B), and identically 75 percent of the activity was trapped as measured by bioassay. The remaining 25 percent of the activity was accounted for as a residual on the implements used for transferring the material. This complete accountability of activity indicates, firstly, that a direct relation exists between the peak area and the attractant, and, secondly, that the attractant passes through the column unaltered. Based on the response of the detector and the recovery, the amount of attractant isolated was calculated to be approximately $28\text{ }\mu\text{g}$. The fact that the chromatogram of the isolated attractant compound shows no peak before or after the attractant peak indicates that the compound has been obtained in a highly purified state. For the detector response used, the sensitivity was of the order of $0.02\text{ }\mu\text{g}$.

Infrared spectra obtained by means

of microspectrophotometric techniques (10) indicate that the compound is aliphatic in nature. The presence of an ester carbonyl is also indicated. Characterization of the attractant is proceeding by means of mass spectrometric analysis of the isolated compound.

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Generation of Electric Potentials by Bone in Response to Mechanical Stress

Abstract. The amplitude of electrical potentials generated in stressed bone is dependent upon the rate and magnitude of bony deformation, while polarity is determined by the direction of bending. Areas under compression develop negative potentials with respect to other areas. Similar results were obtained both in living and dead bone. Removal of the inorganic fraction from bone abolishes its ability to generate stress potentials. It is probable that these potentials influence the activity of osseous cells directly. Furthermore, it is conceivable that they may direct, in some manner, the aggregation pattern of the macromolecules of the extracellular matrix.

Malaligned fractures in children usually straighten in time as new bone is deposited on the concave side of the deformity and old bone is removed from the convex side (1). Similar behavior has been described during healing of fractures in vitro (2). These and other examples indicate that mechanical factors may direct the pattern in which bone is deposited and removed. While the relationship between stress and bone architecture has long

been recognized (3), the mechanisms by which force may influence cellular activity in bone are not completely known. Despite the fact that mechanical factors have been demonstrated to have a direct action on osteogenic cells in vitro (4), other regulatory processes may be in operation. Since bioelectric or direct-current fields have been linked with cellular migration (5), tumor formation (6), morphogenesis (7), and regeneration of amphibian limbs (8), it seemed desirable to investigate whether mechanical deformation could produce measurable d-c potentials in bone. Both mammalian and amphibian bone were employed in our study. Potentials were measured with matched silver-silver chloride electrodes and direct-coupled amplifiers having input impedances ranging from 10^6 to 10^{14} ohms. There was minimal electrode polarization during the procedure.

Initial observations were made on fresh preparations of feline fibulae from which the soft tissues surrounding the central portion of the shafts had been removed by subperiosteal dissection. Care was exercised to prevent drying and to limit artifacts produced by electrode movement during deformation of the bone. With one electrode placed on the posterior aspect and another one opposite it on the anterior aspect at midshaft, stress was applied so that the thin fibula bowed—concave posteriorly. The posterior electrode instantly became negative with respect to the anterior one and remained so, though with a slowly decreasing difference, until the deforming force was removed. The bone immediately returned to its normal shape but the anterior electrode became briefly negative with respect to the posterior, and then returned to a state of isopotentiality. An equal deformation of the bone in the opposite direction produced a reversed polarity of equal magnitude. The amplitude of the potential was dependent upon the rate and magnitude of the bony deformation, while its polarity was determined by the direction of bending. Subsequently, freshly removed specimens of cat fibula, rat femur, and bullfrog tibiofibula (Fig. 1)—fixed in insulated clamps—demonstrated the same phenomena. The potentials generated in these stressed bones apparently were not dependent upon cell viability, since frozen and thawed or air-dried specimens behaved like the fresh prepara-

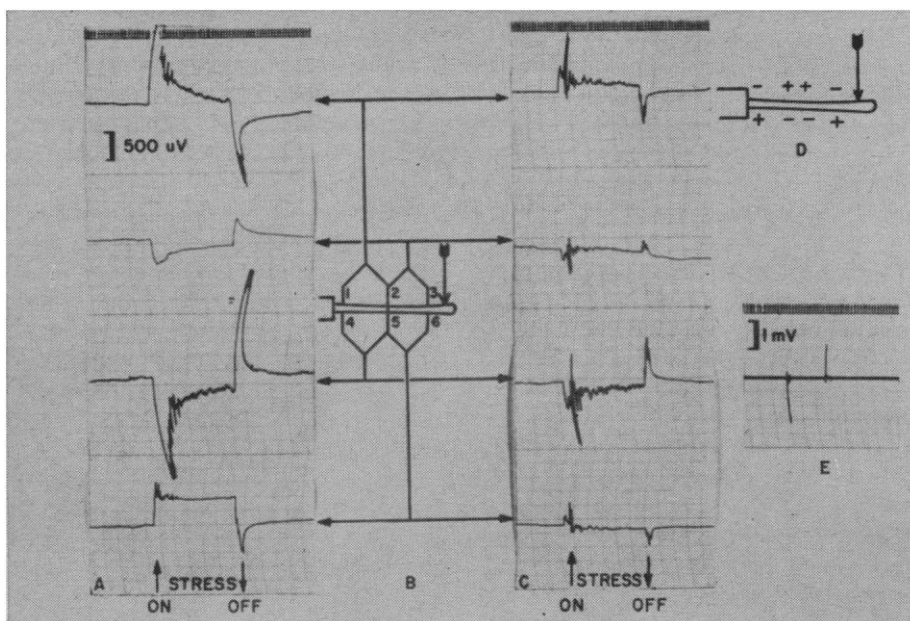


Fig. 1. Electrical response of amphibian bone (tibio-fibula of bullfrog) to bending stress. Electrode placements and connections on bone in clamp are shown schematically at B. Differential inputs were used and the center electrodes (Nos. 2 and 5) are common to two channels. The amplification for record C is the same as that shown for A, and upward deflection of the pen in both indicates a negative potential. The applied stress in A was 30 g, that of C was 15 g. Each mark at top of charts A and C equals 1 second. The potentials developed should be compared to those observed from a quartz piezo-electric crystal with the same instrumentation (E). The crystal produces brief potential changes in one direction on application of stress, and equally brief impulses of opposite polarity on release of stress. The potentials obtained from the bone are similar but decrement much more slowly and show a sustained potential during the time the stress is applied. This implies a persistent charge separation under stress with a charge distribution on the bone surface, as shown schematically at D.

tions, with the exception that the amplitude of the potential was decreased approximately 25 percent.

No potentials were detected when wood, polyethylene strips and fresh tendo Achillis from the cat were deformed in a variety of ways. Decalcified bone lost its capacity to generate d-c potentials in response to stress. When the organic fraction of cat fibula was removed with ethylene diamine, the resultant "anorganic" bone was so fragile that it fractured before significant deformation had occurred, and therefore gave no measurable change in the d-c potential.

It is evident from these experiments that bone develops a negative potential in areas of compression. While the mechanism by which it does so is obscure, two possible explanations may be considered. Since bone is composed of crystals of hydroxyapatite, it has been suggested that piezo-electric properties may be responsible for the conversion of stress to electric stimuli which may affect the neighboring osteogenic tissue (9). The initial potentials obtained from bone on the application of stress were similar to those of a

quartz piezo-electric crystal, but subsequently the potentials in bone decayed much more slowly and were sustained during the time the stress was applied. These results suggest, therefore, that piezo-electric phenomena, while probably present, were not the sole cause of the potentials.

Recently (10), "displacement potentials" were described during bending of rod-like polyelectrolytes of potassium hyaluronate. These potentials were thought to occur when a number of molecules were bent in the same way, displacing significant numbers of free charge carriers from the inside to the outside of the molecules. When the bending ceased and the resulting stress had disappeared, the deformed molecules would tend to straighten, with return of the free charges to their original sites. Although hyaluronic acid has been detected in bone (11), it is questionable whether it is present in sufficient amounts and orientation to produce displacement potentials. The steady state potentials (noted after the initial deflections and throughout the duration of the applied stress) were observed with input impedances as low as 10^6

for as long as 60 seconds. It is therefore inferred that some direct current flow, of low magnitude, was necessary to maintain the potentials. The mechanism responsible for this phenomenon is unknown. Preliminary experiments on charge storage capabilities of bone indicate some solid-state (electronic-conduction) mechanism.

These studies, in themselves, do not establish the relationship between cellular activity in bone and the concentration of charge. However, other investigations (5, 8) have demonstrated that cells are responsive to alterations in d-c fields, and it seems likely, therefore, that similar behavior might be expected of the cells in bone. Experiments currently in progress have already demonstrated the feasibility of directing the pattern of bone growth in vitro with externally applied currents and magnetic fields (12; 13).

Note added in proof: Since submitting this report for publication, it has come to our attention that E. Fulcada and I. Yasuda performed a similar study in 1957 (14). While the techniques employed in our investigation and the results obtained are somewhat different, there are enough similarities that we wish to acknowledge their precedence in this work.

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