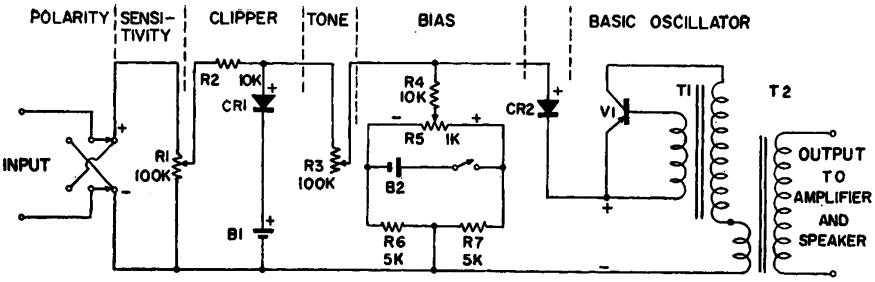

Fig. 1. Schematic diagram of frequency-modulated oscillator: K, 1000 ohms; CR1 and CR2, 1N56 high-conduction germanium diodes; V1, CK 722 p-n-p junction transistor; T1, 1/3 interstage transformer; T2, microphone transformer; B1 and B2, 1.4-v battery.

Furthermore, the power requirements are so low (a 50-μw input gives a 400-cy/sec tone) that the oscillator may be powered directly by the electric signal. Thus, amplitude variations of an otherwise inaudible signal, whether they are periodic or random in nature or even slow changes in a steady level, are translated into tonal variations. The audio-frequency tones produced may be, with experience, of great diagnostic or interpretive value. However, the device was designed as a monitor, since visual patterns are usually easier to study and interpret.

The monitor was designed to be connected across the coils of a recording galvanometer (for example, an electroencephalograph or electrocardiograph) but can be used with any other source providing more than 0.5-v input to the monitor. The output can be fed into a loudspeaker system or high-impedance earphones. The device is simple, self-contained, reliable, and inexpensive (about $10 for parts). More elaborate, but less versatile, heart monitors have been available commercially (*3*).

The basic oscillator gives an 1800-cy/ sec tone at 0.2-v input, a 200 cy/sec tone at 0.75-v input, and a 50-cy/sec tone at 2-v input. Below about 400-cy/sec, the tone is more in the nature of a pulse than the sine wave found at higher frequencies. The load on transformer T2 (Fig. 1) affects the quality of the tone. Polarity is important. If no oscillations are produced when about 1.0 v is applied to the basic oscillator, the leads of the secondary of transformer T1 should be reversed. The diodes and transistor are damaged by heat, so the leads should be kept long, and a hemostat or pair of pliers should be used as a heat sink when soldering.

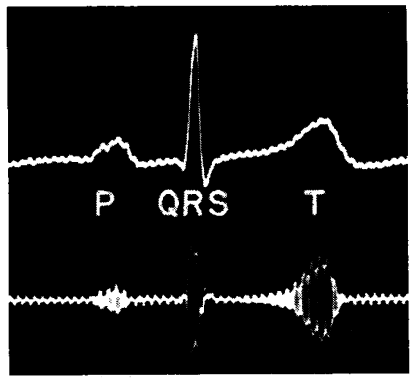

Fig. 2. Oscilloscope tracing of normal human electrocardiogram (top) with output from the audio monitor (bottom). The P wave gave a high-frequency tone followed by the QRS complex, which gave a short, low-pitched tone. The T wave gave a softer, medium-pitched, prolonged tone.

Several refinements were added. (i) A sensitivity control, R1, regulates the input voltage required for a given tone. At maximum sensitivity, 0.5 v gives an 1800-cy/sec tone, whereas 5 v are needed at mid-range, and more than 100 v at nearly zero sensitivity. (ii) A clipping circuit consisting of a 1.4-v mercury battery and a crystal diode, CR1, was added to give the device a roughly logarithmic response. This prevents the suppression of low-level components in a signal with a wide dynamic range. As an example, the QRS component of the electrocardiograph (Fig. 2) has a much higher amplitude than the P and T waves, and thus tends to overpower their tone. The T wave, however, is of particular interest because changes in amplitude or polarity indicate anoxia or heart damage. When the series resistances from R1 plus R2 and from R3 are both greater than 20,000 ohms, the signal going to the oscillator is sharply limited to about 1.4 v. (iii) Variable resistor R3 acts as a tone control for the clipped segments, such as the QRS. Thus, 1.4 v coming from the "clipper" can be set to give a tone from 300 to 2000 cy/sec. (iv) The bias network, R4 through R7 and B2, acts to suppress noise or the effects of power-amplifier imbalance by adding a negative voltage which the signal must overcome to energize the oscillator. By setting it to give a positive voltage, a steady baseline tone is produced which may then be frequency modulated by the signal. A switch is needed on B2 because of the low resistance load, whereas diode CR1 acts as an effective switch for B1. (v) The diode, CR2, rectifies the signal since the oscillator operates only with the polarity indicated (and may be damaged by currents of opposite polarity). For instance, an inverted T wave of an electrocardiogram causes a lack of tone when the bias control is set in the neutral position. A polarity reversing switch in the input circuit is a helpful addition. (vi) The transformer T2 is used to increase the amplitude of oscillator output signal and also to isolate the monitor from the speaker system, since the galvanometers of many direct-writing oscillographs are not at ground potential. Figure 2 shows how this device treats a complex input waveform by converting it to bursts of audio frequencies of different tones and amplitudes.

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29 April 1957

Boron in Morphogenesis of Plant Cell Walls

An intensive study of cell walls in celery (*Apium graveolens* L., var. *dulce* Pers.) grown under different boron levels was undertaken because of the widely recognized effects of boron nutrition on carbohydrate metabolism in plants (*1*). In addition, the extensive literature on boron nutrition includes very little information on cell-wall structure. A large body of evidence shows that, under boron deficiency, carbohydrates accumulate in the plant and in some cases new carbohydrates may be formed (*2*). There is also evidence that boron facilitates the translocation of carbohydrates in the plant (*3*, *4*), although a recent report (*5*) does not support this conclusively.

Three varieties of celery were grown in Hoagland and Arnon's solution 2 (*6*) with boron levels modified to range from 0.50 ppm (normal) to 0.00 ppm. Analyses showed that the boron content of the celery was markedly changed by the treatments. The boron content (dry-weight basis) of the petioles of Dwarf Golden Self Blanching, for example, was 36 ppm when the plants were grown at 0.50 ppm and 13 ppm when they were grown at 0.01 ppm. Measurements of cell-wall thickness and observations on

Table 1. Collenchyma cell-wall thickness as affected by the boron supply in the nutrient solution in three varieties of celery.

Variety	Wall thickness (μ) at contrasting levels of boron	
	0.50 ppm (normal)	0.00 ppm (deficient)
Dwarf Golden Self Blanching	8.2	2.8
Utah 15	8.0	2.3
Utah 10-B	9.4	4.1

the fine structure were made on sections 2 or 3 μ thick from material imbedded in a water-soluble medium (polyethylene glycol). After the sections had been stained, an aqueous mounting medium (*7*), slightly modified from the previous formula, was used in preparing slides. These histological methods, especially for parenchymatous tissues, avoid the cell-wall shrinkage caused by the paraffin techniques. A polarizing microscope and microchemical and solubility tests were used in the study of cell-wall constituents. This study was restricted to material collected from the mid-level of petioles that were estimated to have just completed length growth. Thus, observations were made on mature petioles that had grown under various levels of boron supply during their major period of development. Observations were further restricted to three tissues: (i) the collenchyma immediately under the epidermis of the ribs on the abaxial side; (ii) the larger, more peripheral elements of the phloem parenchyma in the bundle cap; and (iii) the ground parenchyma.

At a normal level of boron nutrition (0.50 ppm), the thicker portions of the common wall between two collenchyma cells in all three varieties of celery are about 8 to 9 μ thick. Under extreme boron deficiency (0.00 ppm), however, the thicker portions of the walls of most collenchyma cells are only about 2 to 4 μ thick (Table 1; note extreme right portions of Fig. 1). The effect is more or less directly proportional to the boron supply.

Table 2. Phloem parenchyma cell-wall thickness as affected by the boron supply in the nutrient solution in three varieties of celery.

Variety	Wall thickness (μ) at contrasting levels of boron	
	0.50 ppm (normal)	0.00 ppm (deficient)
Dwarf Golden Self Blanching	1.0	3.5
Utah 15	0.81	2.2
Utah 10-B	1.2	2.4

In the phloem parenchyma of all three varieties, the cell walls become much thicker under boron deficiency. At 0.50 ppm, the common walls between cells are about 1.0 μ thick, whereas at 0.00 ppm the walls range from about 2 to 3.5 μ thick (Table 2). This response is more or less inversely proportional to the boron supply. Similar striking changes in cell-wall thickness occur in the ground parenchyma. The effect on these two tissues is in marked contrast to the response shown by most of the collenchyma cells. The level of boron in the plant apparently affects the rate of carbohydrate condensation into wall material.

Some collenchyma cells in two of the varieties (Dwarf Golden Self Blanching and Utah 10-B) exhibit a very special response to boron deficiency. The cells that are affected are located at the periphery of the collenchyma strands, although more deeply situated cells in the strands may also show the response. Whereas normal collenchyma cells are characteristically thickened at the "corners" (that is, at the three- or four-rayed vertices of the contacting walls), the specially affected cells have a massive, uniformly thick inner band of wall material (Fig. 1). This is a very basic change in the way the cell wall is laid down. The thickening phenomenon in the specially affected collenchyma cells appears to be similar to that occurring in the adjacent ground parenchyma and the phloem parenchyma, except that the thickening in the latter two tissues is not as great.

In normal material the walls are very fine-grained. The cells walls of boron-deficient tissues, however, are much coarser (Fig. 1). Measurements of the light- and dark-staining lamellae in the unswollen walls of the collenchyma of all three varieties showed that in normal collenchyma the lamellae are about 0.30 μ thick, whereas in boron-deficient material their width is about 0.20 μ. Counts were made of the number of lamellae in collenchyma cell walls swollen by microchemical treatments. Normal collenchyma contains about 60 light- and dark-staining lamellae, whereas boron-deficient collenchyma has about 20. These differences in the fine structure of the walls suggest that boron affects the process of carbohydrate condensation into wall material.

The response of most of the collenchyma to a low boron supply suggests that its walls fail to thicken because of a deficiency of carbohydrates. Since the ground parenchyma and phloem parenchyma in the same sections become much thicker walled, it appears that carbohydrates from the major source, the phloem, are condensed in these intervening tissues before they reach the more peripheral collenchyma. If this interpretation is correct, the histological evidence presented here supports the hypothesis of Gauch and Dugger (*3*) that boron facilitates the translocation of carbohydrates in the plant.

Frequent comments occur in the literature (*8*) that mineral nutrition affects the development of the form of the plant —that is, its morphogenesis. This view is based in part on the fact that a number of mineral elements or conditions are reported to affect the walls. Though little detailed information is available on the relationship of mineral nutrition to cell-wall differentiation, the work of Schneider (*9*) provides an example of

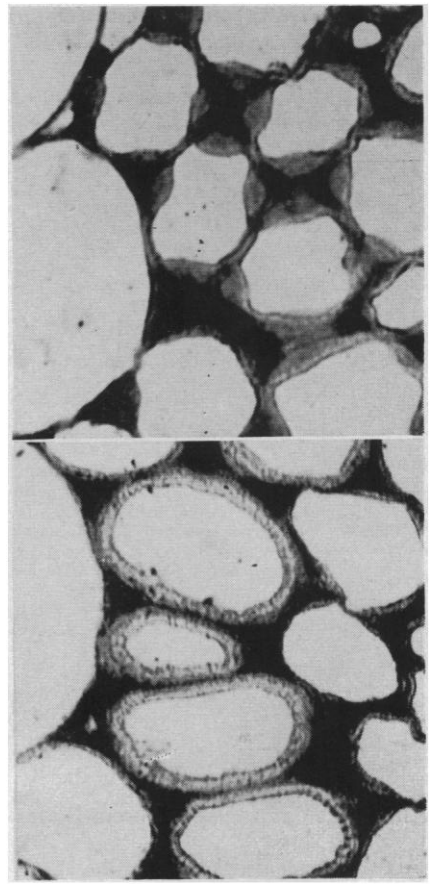

Fig. 1. Transverse sections of celery petioles showing normal and boron-deficient collenchyma tissue near the margins of collenchyma strands in the variety Dwarf Golden Self Blanching. Portions of ground parenchyma cells are on the left side of the photographs. (Top) Normal tissue grown at 0.50 ppm B, showing characteristic collenchyma wall thickenings mainly at the "corners" of the cells. (Bottom) Affected tissue grown at 0.00 ppm B, showing the reduced thickness of the walls in right portion of the photograph and the marked difference in the organization of the walls in the cells at the margin of the strand. × 730.

studies in which this subject is considered. Other agents besides minerals are involved in cell-wall differentiation. Jacobs (*10*), for example, has recently concluded that auxin is a limiting factor in the differentiation of xylem cells. Our evidence suggests that boron is one morphogenetic agent affecting the differentiation of cell walls. How this action may be mediated by boron is not understood. A close involvement of boron in cell-wall differentiation, however, is suggested by the fact that it very likely complexes with a number of polyhydroxy compounds in the plant, such as various sugars and pectic materials (*11*), which become part of the cell-wall substance.

Intensive studies of the cell walls in relation to boron nutrition show that the normal pattern of cell-wall differentiation is profoundly changed by boron deficiency. This fact and the relationship of boron to carbohydrate metabolism implicate boron as an agent in the morphogenesis of plant cell walls (*12*).

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25 April 1957

Amino Acids in Fossil Human Bone

In a recent investigation of the chemical constituents of fossil human bone conducted in our laboratory, it was observed (*1, 2*) that bones of great archeological age may contain appreciable quantities of organic nitrogen. The significance of these findings for dating prehistoric bone has been discussed elsewhere (*2, 3*). It is highly probable that the source of this nitrogen is the original proteins, as suggested by Abelson (*4*). If so, a question of interest is: How many of the constituent amino acids are able to retain their chemical individuality under the conditions attending archeological preservation.

Table 1. Dates of fossil bone samples.

Designation	State and culture period	Date before the present by C^{14} analysis or tree-ring count (yr)
UK3	Kentucky, Archaic	4900–5300
UK25	Kentucky, Adena	1170–1510
S76	New Mexico, White Mound	1175
AP692	New York, Frontenac	4370–5385
8450-1	California, Middle	1880

Each bone was first completely hydrolyzed by hydrochloric acid. Then aliquots of the hydrolyzate were analyzed by two-dimensional paper chromatography. This made it possible to identify the presence of traces of amino acids. As a control, we first examined fresh human femurs secured from autopsy and established the presence of the following amino acids: glycine, alanine, serine, valine, leucine, isoleucine, phenylalanine, tyrosine, cysteic acid, proline, hydroxyproline, aspartic acid, glutamic acid, histidine, arginine, lysine and methionine-sulfoxide. This list agrees substantially with that published by Eastoe (*5*) for fresh bone.

We next investigated a series of 20 fossil human and three fossil animal bones, representing a wide time span from relatively recent to archeologically very old. In bones exposed to burial for comparatively short periods, most of or all the amino acids found in fresh bone are detectable in nearly normal amounts. Some of the samples falling within this classification are shown in Table 1.

In bones the age of which appears to be definitely greater than those shown in the table, the constituent amino acids begin to disappear. We have as yet not developed the quantitative analysis to the point where it is possible to set forth the details of an orderly progression of depletion or retention. Nevertheless, certain amino acids evidently persist in all but the very oldest specimens. Thus, sample 6075 (site FRe-48, California, very ancient) contained only aspartic acid. A human bone, early post-Pleistocene from site LAn-172, California, contained aspartic acid, glycine, and glutamic acid. A mammoth bone from Melbourne, Fla. (site Bre-44, very ancient) and a human bone from site SJo-142 (Early culture period, central California) both contained aspartic acid, glycine, and glutamic acid together with a few other amino acids which differed between the two samples. Finally, a human bone and a horse bone from Melbourne, Fla. (site Bre-44) both gave tests showing no amino acids whatever.

The preliminary results reported here therefore suggest the conclusion that decomposition of protein in buried bones proceeds extremely slowly over many thousands of years but tends to release in the process certain amino acids while retaining certain others with great tenacity (*6*).

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18 March 1957

Behavior of Light- and Dark-Reared Rats on a Visual Cliff

From the 18th century to the present, the empiricist and the nativist theories of depth perception have been vigorously debated. One experiment aimed at resolving the dispute is Lashley and Russell's (*1*), in which rats reared in darkness jumped to a platform from a stand placed at a variable distance from it. The force of the jump was found to be graded in accordance with the distance of the platform. This is evidence for nativism. But, since the tests with graduated distances were not given until the rats' third day in the light, and after pretraining, the conclusion was not indubitable. Confirmation by another technique is desirable and has been provided in the experiment described in this report (*2*).

A technique of testing for visual depth perception which involves no pretraining at all—the "visual cliff"—was developed. It is based on the assumption that, given a choice, an animal will avoid descending over a vertical edge to a surface which appears to be far away (*3*). The apparatus (Fig. 1) was constructed of two thicknesses of glass (24 in. by 32 in.), parallel to the floor and held by metal supports 53 in. above it. A board (4 in. wide, 24 in. long, and 3 in. high) extended across the glass, dividing it into two equal fields. On one side (the "near" side), patterned wallpaper was inserted between the two sheets of glass. Through