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Nonrandom Bioelectrical Signals in Plant Tissue

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ABSTRACT

The results of investigations on nonevoked bioelectrical activity in the India-rubber tree (*Ficus elastica*) are presented. Metal electrodes inserted into the plant issue were used as the ionic-to-electronic conduction converting elements. Nonevoked pulse bursts were observed with amplitudes in the 10 to 200 microvolts range. An upper limit value of the cell refractory period has been estimated from the maximum pulse frequency observed.

The existence of evoked action potentials in plants due to a stimulation of the cell depolarization-repolarization process is well established. The concept of stimulation covers a broad range of interactions between the cell and its environment. In some cases the act of observation might be considered a stimulation in itself because of the interaction present during the observation. The general bioelectrical measurement situation is influenced by the not negligible interaction between the measurement apparatus and the object under investigation.

In the present work, measurements have been made of nonevoked bioelectrical signals in the tissue of the India-rubber tree (*Ficus elastica*). Nonevoked in this case means that no stimulus was applied to the plant except for the presence of the electrodes in the petioles and the bias current of the amplifiers which is less than 10^{-10} amp. Bursts of pulses with well defined and rather constant amplitudes have been found to be produced by bioelectrical generators located in the plant tissue. Pulse burst lengths ranging from 30 sec to 25 min have been observed. The frequency of occurrence of the pulses within the burst is in the 0.5 to 200 pulses per minute range. Great effort was made to ensure that pulses generated by sources external to the plant were not mistaken for signals generated in the plant. Because of the electrode arrangement used, the pulses observed are assumed to represent the cellular action potential produced by groups of cells, analogous to the extracellular action potential as defined in medical electrophysiology (3). This assumption is substantiated by the fact that, while the single plant cell polarization-depolarization action potential has an amplitude of about 70 mv (6, 14), the potentials measured in the course of the present work were all in the 10 to 200 μv range. A difference in amplitude of the same order of magnitude between the intra- and extra-cellular action potentials is also found in medical electrophysiology (11).

As in animal tissue, the electrical activity in plant tissue is based on ion transport mechanisms (8). Due to this circumstance, it is necessary to make a conversion from the ionic conduction present in the tissue to the electronic conduction which occurs in the measuring circuit, in order to measure electrical effects in the plant tissue. This conversion is accomplished at the tissue-electrode interface. The electrodes should preferably perform this conversion without disturbing the ionic concentrations or permanently damaging the plant tissue. In the present work, metal electrodes made of stainless steel and gold were used. Tests showed that electrodes made from the latter metal produced relatively stable and noise-free operation.

It is generally assumed that plants do not usually require for their functions any faster transfer of information than that provided, for example, by auxin translocation and other chemical transport mechanisms (9). The results of the present work tend to show that short range information transfer can occur in plant tissue at much higher speeds than those provided by the diffusion of chemical constituents. Within the framework of the present investigations, no attempts have been made to interpret the physiological significance of the pulse bursts observed.

A part of the multitude of potential difference measurements that have been performed on plants are reported on in references 1, 2, 5, 10, and 12. Some of these have concerned the measurement of the bioelectrical response of a single cell to the application of a stimulus either physical or chemical. Single cells of *Nitella translucens* and of *Chara* have been investigated intensively. These cells produce bursts of pulses as a result of the application of a stimulus. This stimulus may be a simple one such as simply punching a hole in the cell (1) or sending an electrical current through the cell. Also, stimulated action potential generation in higher plants by means of an electrical current passing through the tissue has been investigated (12).

The existence of static cell transmembrane potentials in higher plants is well established (4, 6, 12). The static transmembrane potential is the resting potential of a polarized cell which for large cells can be measured across the membrane by means of a high impedance voltmeter. Measurements of the transmembrane potential have been performed (6) by means of the combined application of chemical analysis and the Nernst equation,

$$E_{io} = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

where E_{io} is the transmembrane potential, R the gas-constant, T the absolute temperature, z the charge on the ions in question, F the Faraday constant, and C_o and C_i the ion concentrations outside and inside the cell membrane, respectively. However, it is impossible by means of this technique to measure short term variations such as the action potentials generated by the cell depolarization-repolarization process.

In the present series of electronic measurements, performed primarily on the India-rubber tree, a relatively steady production of nonevoked pulse bursts has been observed. With the same specimen and a stationary electrode placement on it, the over-all electrical activity has shown no signs of attenuation or pulse amplitude change for several weeks of observation. The presence of this nonevoked bioelectrical activity is in a sharp contrast to the results of measurements, mostly performed on

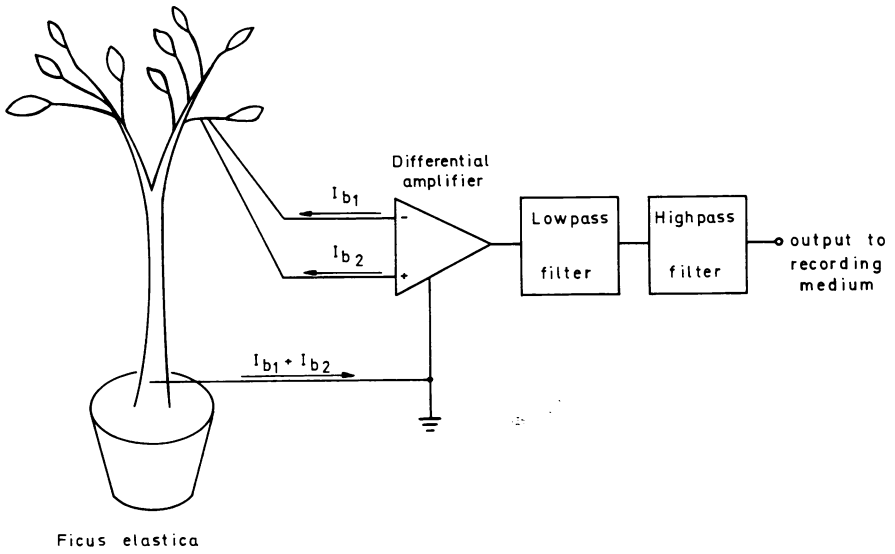

FIG. 1. Simplified schematic of the experimental setup.

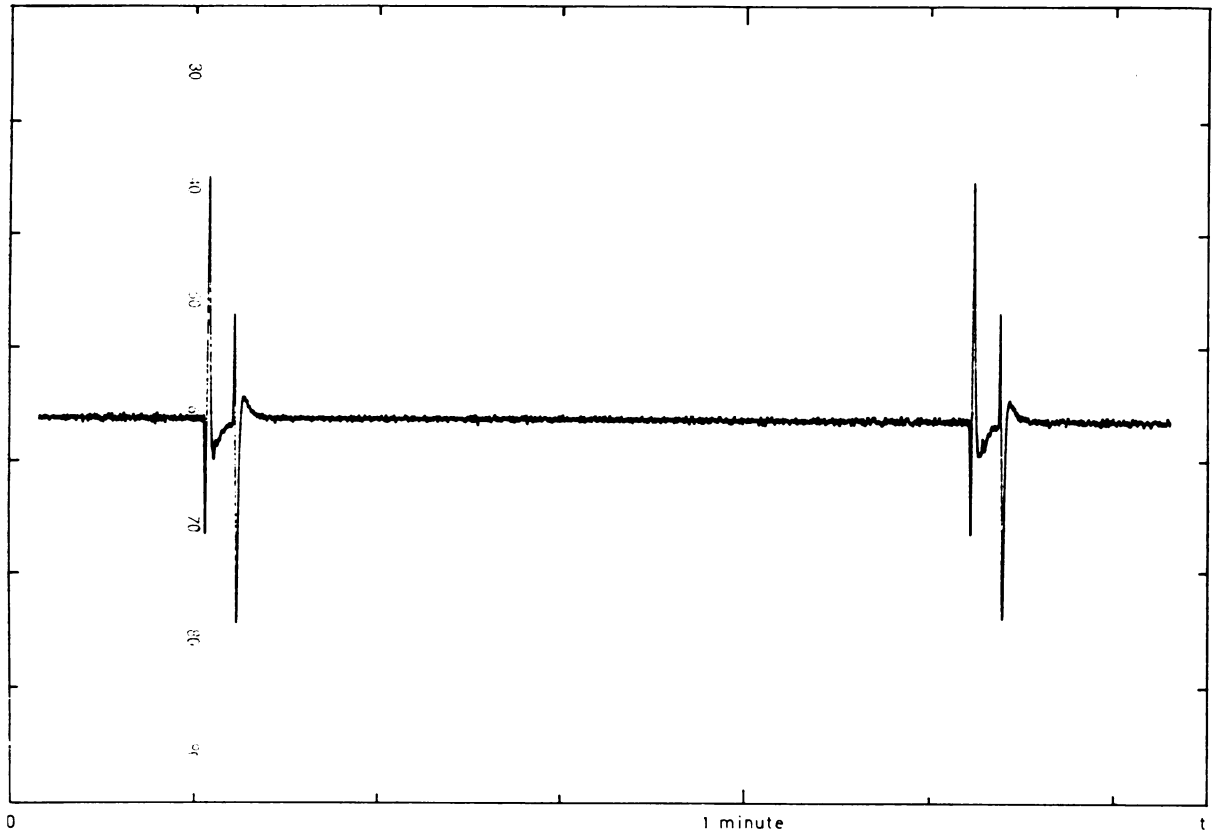

FIG. 2. Two pulses with a 150-μv peak-to peak amplitude. They were members of a burst containing 18 pulses, all having similar appearance.

single cells, reported upon earlier. According to the results of the present work, the *Ficus elastica* plant produces bioelectrical signals intermittently as a part of its normal life. It seems that the observed activity does not have any direct correlation to parameters which otherwise are important in the function of the plant, such as light intensity.

MATERIALS AND METHODS

The experimental setup is described in great detail in reference 7. In Figure 1 is shown a simplified diagram of the instrumentation used. Due to the high CMRR[1] of the differential amplifiers employed, it was found superfluous to shield the plant in order to discriminate against external sources of interference signals. In practice, two identical amplifier systems with associated electrodes placed on different parts of the plant were used. By this method, externally generated interference is identified by its simultaneous presence in the output signals from both amplifiers. Ink recorders and a dual-trace

[1] Abbreviation: CMRR: common mode rejection ratio.

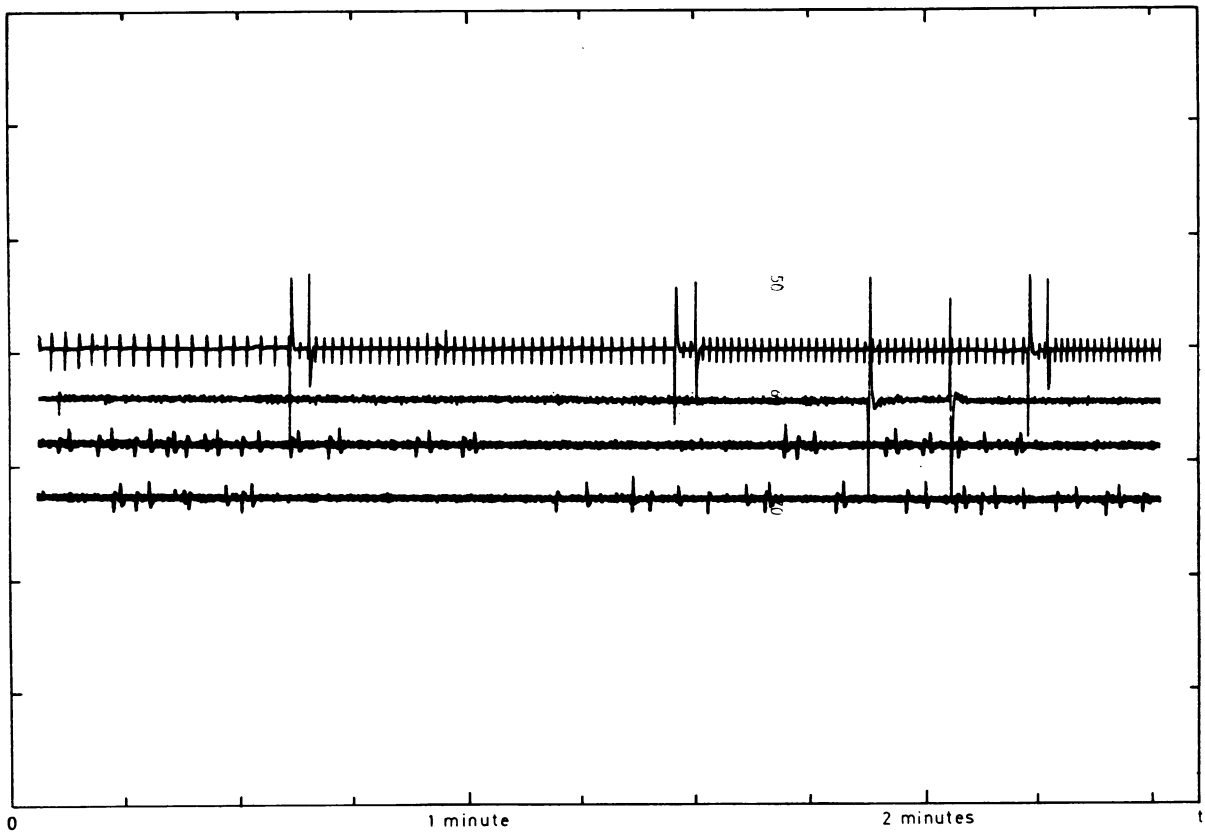

FIG. 3. The upper trace displays frequency shifts with a larger pulse preceding each shift. The lower three traces show the activity when no pulse bursts are present. The upper trace is recorded with 100 μv/div, while the three lower traces are recorded with 40 μv/div.

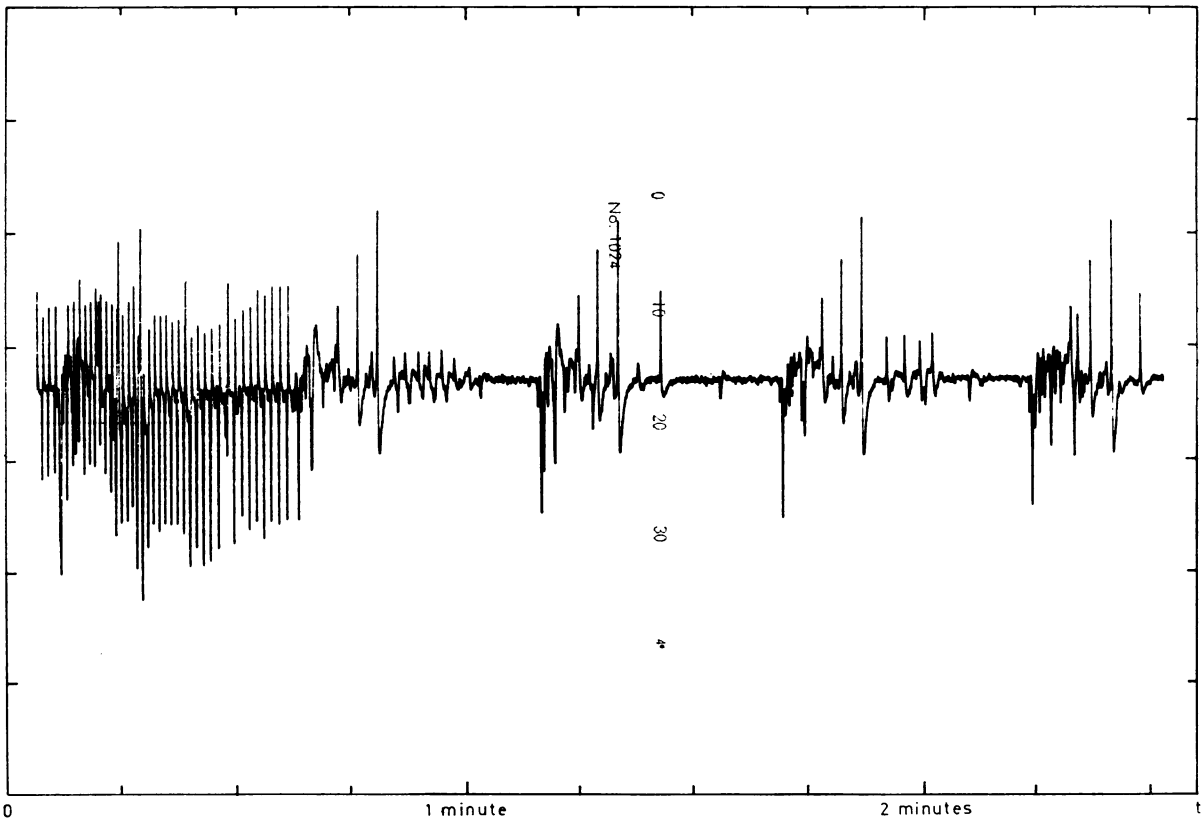

FIG. 4. This recording shows an abrupt shift from about 100 pulses per minute to a much lower frequency.

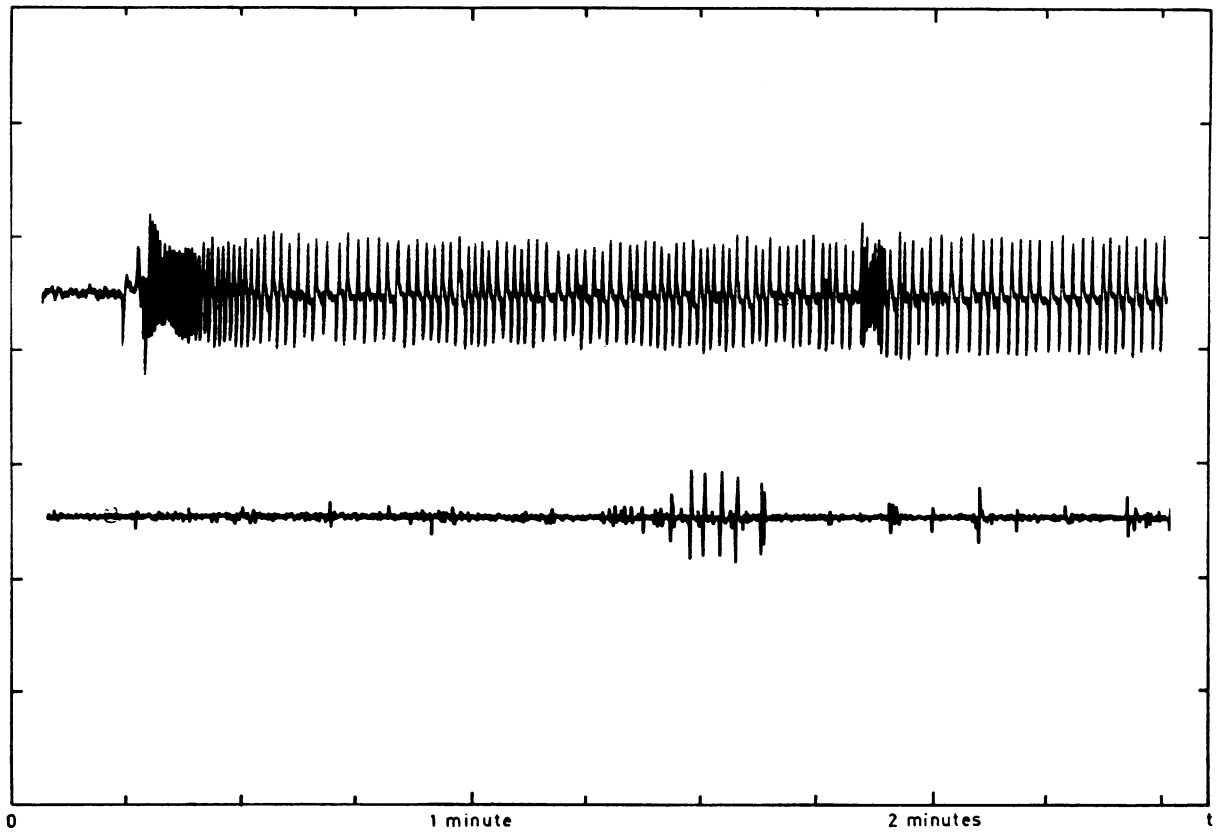

FIG. 5. The upper and lower traces are the simultaneous recordings of the bioelectrical activity measured at different places on the same plant. Excellent isolation between the channels is observed. Vertical sensitivity is 80 μv/div in both traces.

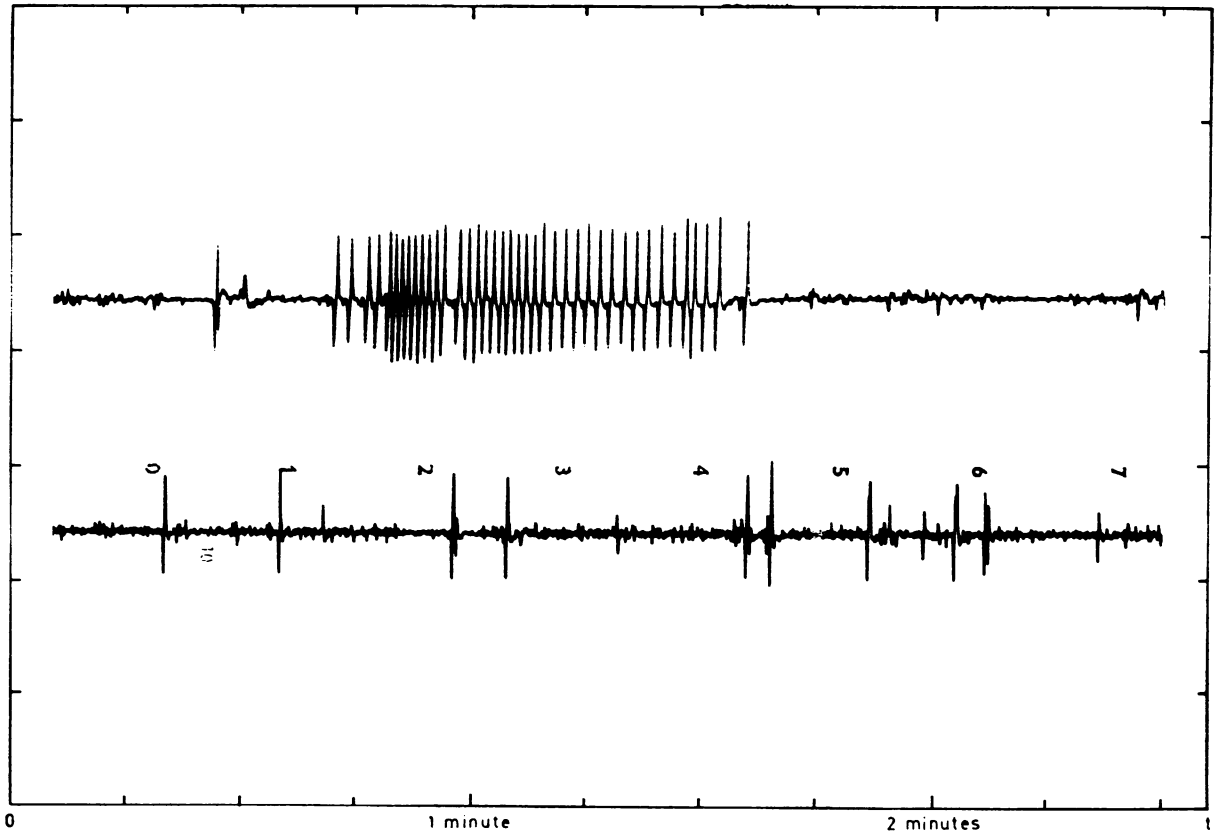

FIG. 6. The conditions of recording are the same as in Figure 5.

storage oscilloscope were used as the recording media. The electrodes were made from 0.4-mm diameter gold leads. These are inserted in the petioles close to the leaves as indicated in Figure 1. The electrodes were connected to coaxial cables by means of small coils of 0.1-mm copper lead to avoid mechanical loading of the electrodes by the coaxial cables, which in turn were fastened to the more rigid parts of the plant. The electrode-tissue interface potentials stabilized within 2 hr after insertion of electrodes in the tissue. No abnormalities in the development of the plant due to the presence of the electrodes were observed.

The plant under investigation was kept from exposure to direct sunlight in order to avoid the possibility of thermally induced electrode-tissue potential variations.

EXPERIMENTAL RESULTS

In Figures 2 to 6 are shown selected samples of the recordings made during a 2-month observation period. They are representative for the repetitive signals observed, which are interlaced by periods of apparently more or less random activity. The amplitudes of the pulses were remarkably constant within each burst and had an over-all range of 10 to 200 μv. The sum of noise generated in the amplifiers, at the electrode-tissue interfaces, and in the plant tissue was below 5 μv peak-to-peak when a lower cutoff at 0.5 Hz and an upper cutoff at 50 Hz were used. The bias current of the preamplifiers used for recording the action potential pulse bursts shown in Figures 2 to 6 was less than 100 pamp.

The shape of the pulses shown is determined by the bioelectrical signal characteristics and the pulse response of the recording equipment used. The ink recorders used have a very limited bandwidth, from zero to about 10 Hz, and displayed some overshoot with large deviations, which to some extent influences the shape of the pulses shown. The detailed characteristics of the signals are best observed by means of a storage oscilloscope, using a sweep rate of about 0.5 sec/cm. Also, an oscilloscope with a persistent phosphor, *e.g.*, a P7 phosphor, will operate satisfactorily.

The highest pulse repetition frequency observed was about 200 pulses per minute, which indicates a refractory period not longer than 0.3 sec for those cells participating in the generation of the observed bioelectrical signals. Whether this period should be considered the absolute or the relative refractory period remains to be settled, as these two terms have until now only been applied in the case of evoked responses in plants (14).

CONCLUSIONS

A physiological interpretation of the observed bioelectrical activity is clearly needed. The first step towards this would be to arrange for long term measurements of the plant bioelectrical activity in which correlations to all relevant parameters were made. During the present observation period of 2 to 3 months, no clearcut correlation which could stand firmly on the basis of the experimental results was found. During limited periods of time, a marked trend towards a circadiurnal rhythm was observed. It was in no case observed that the bioelectrical activity was unusually high during those periods of the day and night when man-generated electrical noise is at a high level. The measurements were performed at a site where the environmental man-made electrical activities are at a low and well controlled level.

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