BIOELECTRICAL ACTIVITY OF HIGHER PLANTS

by

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ABSTRACT

Using surface located calomel electrodes and high input impedance amplifiers, phytochrome-mediated potential changes at the wheat coleoptile tip were measured. Potential changes appear to be one of the primary phytochrome-induced events. Red light induced a positive change (2-7 mV) in potential at the coleoptile tip, and far red reversed the effect. All parts of the coleoptile seemed to be equally perceptive and IAA did not appear to be directly involved in the process.

Extracellular tungsten or Ag/AgCl electrodes and high input impedance amplifiers were used to measure electrical activity of light-grown plants. *Cucurbita* plants showed 4 types of spontaneous electrical activity, viz. trains of small (0.25-4 mV) repetitive signals resembling action potentials which were detected mostly in young growing parts; "irregular" signals of varying shapes, sizes and polarities; complex activity which seemed to be a result of a superposition of the asynchronous activities of many cells; and rare, large (8-35 mV) "solitary" signals which appeared to be a result of the synchronous firing of many cells. The spontaneous electrical activity does not appear to be related to phloem transport and is not affected by light conditions, IAA, ACh or ABA.

One or more, large (10-35 mV), propagating action potentials were induced as a result of a heat stimulus to a leaf or the roots, which could spread throughout the plant, but had no effect on the rate of respiration of the neighbouring undamaged leaves.

Extracellular and intracellular potential measurement techniques were used to study the effect of various factors on membrane

potentials and action potentials. KCl treatment (0.12–1.0 M) of the roots of an intact *Cucurbita* plant or isolated stem sections induced a number of large (10–45 mV), non-propagating action potentials which could be detected at a distance due to electrotonic conduction. A possible mechanism of electrotonic conduction has been presented. The effect of KCl seems to be only partly osmotic, the major effect probably being due to passive diffusion of K^+ into the cells. Non-propagating action potentials were also induced by a sudden increase or decrease in osmotic pressure around the root, ascorbic acid (100 mM) or KCN (10 mM) treatment. The sole requirement for the generation of these action potentials seems to be a depolarization of the cell membrane potential beyond a threshold level. Anoxia and IAA (10^{-5} M) caused a small depolarization of the membrane potential but insufficient to generate action potentials. IAA (and to some extent ACh) seem to increase the membrane permeability to K^+ and to enhance the production of KCl-induced action potentials. The non-propagating KCl-induced action potentials in the roots did not have any major control over the rate of CO_2 exchange of the leaves. A possible role of the non-propagating action potentials in the roots in osmoregulation has been discussed.

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ABBREVIATIONS

AA	Ascorbic acid
ABA	Abscisic acid
ACh	Acetylcholine
Ag/AgCl	Silver, Silver Chloride
ATP	Adenosine 5'-triphosphate
2,4-D	2,4-dichlorophenoxyacetic acid
DNA	Deoxyribonucleic acid
FAP	6-Furfurylaminopurine (Kinetin)
FC	Fusicoccin
FR	Far red light
GA	Gibberellic acid
IAA	Indole-3-acetic acid
Pfr	Far-red-absorbing form of phytochrome
Pr	Red-absorbing form of phytochrome
R	Red light
RNA	Ribonucleic acid
SE	Standard error
2,4,5-T	2,4,5-trichlorophenoxyacetic acid

1. INTRODUCTION

1 INTRODUCTION

Electrical signals in plants which resembled animal nerve impulses were first detected in plants over a hundred years ago (Burdon-Sanderson, 1873; Darwin, 1875). Since that time, it has become more and more apparent that these represent only a part of the complex electrical phenomena which occur in all living organisms and which can govern their growth, differentiation and response to stimuli.

Living cells contain aqueous phases which are separated from each other and from the external medium by membranes. The transfer of any ionizing substance during passive or active transport across these membranes may result in charge separation and a consequent setting up of transmembrane potential gradients. In practice the inside of the cell is almost always more negative than the external solution by up to 150 mV. The potential gradients across the membranes may affect the diffusion of other ions and charged molecules provided they are free to move independently. Even active transport via a carrier complex will be affected, providing the complex is not neutral. The transmembrane electrochemical gradient, set up by H^+ pumps has been suggested to provide the driving force for the transport of many neutral molecules, e.g. the cotransport of sucrose and hydrogen ions by *Ricinus* cotyledons (Hutchings, 1978). The inter- and intracellular transport of many other organic substances which carry charges (e.g. organic acids, amino acids, adenosine phosphates etc.) may also be influenced by the transmembrane potentials. Thus transmembrane potential gradients will have a profound effect on metabolism since the cell is a complicated network of membrane

barrier systems, and many biochemical processes will depend on the access of the substrates to enzymes which may occur in different compartments.

Apart from these obvious effects, the transmembrane potentials might also have a significant influence on the activity of the membrane-bound enzymes. Despite the fact that an average membrane potential is only a fraction of a volt, because the membrane is so thin, the voltage gradient across it is of an immense magnitude (ten million volts per metre). This would be expected to have a considerable effect on the conformation of the enzymes within the membrane due to orientation of charged groups which, in turn, would be expected to affect the enzyme's activity. Chloroplast ATPase has already been shown to be controlled in this way (Graber et al., 1978) and there may be many other membrane-bound enzymes which are similarly controlled.

Some investigators have linked these electrical phenomena to the control of growth and development of many plants and animals. On the basis of observations on L-strain mouse fibroblasts and a number of other reports, Cone (1970) suggested that a significant correlation exists between the level of electrical transmembrane potential difference in cells and the level of their mitotic activity. In addition, differences in the membrane potential in different regions of the cell allow currents to flow, the direction of which are thought to influence the direction of growth and cellular polarity. In many animal eggs, a steady current was found to enter the animal pole while leaving the vegetal pole or equator during their development (Robinson, 1979; Woodruff and Telfer, 1974). Such external and internal currents have also been found in regenerating newt limbs (Borgens et al., 1977), and healing bone fractures in

frog, rabbit and humans (Friedenberg and Brighton, 1966; Becker and Murray, 1970). Furthermore, artificially applied currents have been shown to induce partial regeneration in amputated frog limbs (Smith, 1967). Similar analogous systems have been shown in a number of plant cells and organs. The unfertilized egg of *Fucus* has a small (-30 mV) resting potential (Weisenseel and Jaffe, 1972), which rises rapidly to -70 mV on fertilization. Parthenocarpic activation by a variety of environmental factors also induces the increase in the membrane potential. Similar hyperpolarization also occurs in lily pollen grain following wetting (Weisenseel, 1977). The tip growth in these cells has been shown to be preceded and accompanied by a leak of calcium ions causing a current to flow into the region of the growing tip (Jaffe et al., 1974; Jaffe et al., 1975). Similarly, the differentiation of *Acetabularia* appears to be associated with electrical phenomena. Enucleated stalk segments of *Acetabularia* which have been morphologically depolarized by a light treatment will regenerate a new cap with nearly equal frequency at either end but thirty hours before the cap regeneration begins, 100-sec-long current pulses begin to enter one end and it is this end which finally develops the cap (Novak and Bentrup, 1972). Recently Weisenseel et al. (1979) have shown proton currents in growing roots of barley, suggesting a similar phenomenon in higher plants. On the basis of these and many other similar examples in plants and animals, Jaffe and Nuccitelli (1977) concluded that developing systems generally drive steady ion currents through themselves and thus produce significant internal fields. They suggested a role of these fields in the electrophoretic migration of charged components along cell membranes and the control of their distribution.

Electrical fields have also been found to be associated with growth responses involving IAA. Using vibrating electrodes, Grahm (1964) and Woodcock and Hertz (1972) showed that placing the corn coleoptile horizontally, induced potential differences of about 70 mV across them, with their lower (and faster growing) side being positive. Similar electrical fields have been reported to be associated with phototropic responses (Johnsson, 1967). These geoelectric and photoelectric potentials have been shown to be a consequence of gravitation - or light-induced auxin movement. Jenkinson and Scott (1961) reported that the otherwise steady electrical field in a bean root could be forced into an oscillatory state by subjecting it to rhythmic changes in auxin concentration. A similar oscillatory bioelectric behaviour was observed in *Avena* coleoptile induced as a result of a change in the downward flux of IAA (Newman, 1963). The electrical disturbances travelled down the coleoptile at the speed of auxin transport (Newman, 1965), thus confirming the close association between the two. The electrical fields produced due to variable auxin distribution can be explained on the basis of the evidence obtained from intracellular potential measurements (Etherton, 1970; Cleland et al., 1977), which suggested that applied auxin causes hyperpolarization. However, it should be noted that Goring et al., (1979) observed an IAA-induced depolarization in short-term (a few minutes) and suggested that it is the initial depolarization which activates the H^+-efflux across the plasmalemma which in turn controls cell wall plasticity and growth (Cleland, 1975). It is possible that the hyperpolarization which others have observed is a consequence of this H^+-efflux. IAA is also known to affect the cell permeability to many ions, specially K^+ (Ilan, 1973) and Cl^- (Rubinstein and Light, 1973). Thus, it seems that IAA plays

a major role in the production of electrical fields in the plants and therefore, in any investigation into the bioelectrical activity, its importance should not be overlooked.

In this context it should be noted that there are many other growth regulators which are known to affect ion transport and therefore presumably have some effect on the electrical activity. Ilan (1971) and Ilan et al. (1971) observed a change in K^+/Na^+ selectivity in favour of K^+ uptake under the influence of kinetin in leaf discs and expanding cotyledons of *Helianthus*. Cram and Pitman (1972) and Van Steveninck (1972) showed that ABA affects K^+ and Cl^- fluxes. Kinin (and ABA) also play a key role in regulation of stomatal opening (Livne and Vaadia, 1965; Jones and Mansfield, 1970) which depends on rapid changes in concentration of K^+ and an accompanying anion in the guard cells. Van Steveninck (1976) showed that GA_3 increased the leakage of K^+ and Na^+ from beet root discs by increasing membrane permeability. Thus, in addition to IAA, the other phytohormones may also have some effect on the membrane potentials and electrical fields.

Perhaps related to the hormone responses are changes in electrical potential resulting from stimulation of the phytochrome system. The earliest phytochrome-controlled electrical phenomenon reported concerns the surface adhesion properties of barley and mung bean root rips. Tanada (1968a, b) showed that adhesion of root tips to a negatively charged surface was under phytochrome control, and predicted that it involved rapid changes in the surface charge of epidermal cells of the root tip. Jaffe (1968) further demonstrated by direct electrical measurement that the predicted electrical changes did indeed occur. Red light induced a positive charge at the root tip and far red light had the reverse effect. Jaffe (1970)

suggested that red light increased the content of acetylcholine in the tissue which mediates the final response by increasing H^+-efflux. Phytochrome, which can control the leaf movements in nyctinastic plants, was also shown to induce rapid potential changes in the motor cells followed by K^+ fluxes causing turgor changes leading to movement (Racusen and Satter, 1975). In the oat coleoptile, red light induced a rapid increase of as much as 10 mV in the potential near the coleoptile tip relative to its base (Newman and Briggs, 1972); again far red light had the reverse effect. Clearly, further investigation into this interesting phytochrome-mediated electrical phenomenon is required to show its physiological role and how it interacts with other electrical phenomena such as those induced by auxin.

A transient fall in the transmembrane potential has been termed as an action potential. Action potentials seem to be an almost universal phenomenon in biomembranes. Besides the classical reports of action potentials in the nerves and muscles in animals, an extremely diverse range of plants, from algae to angiosperms, have been found to generate action potentials. However, they seem to differ in different systems both in duration (for animal cells, in the range of milliseconds; most plants, in seconds and *Neurospora* and *Acetabularia*, in minutes), and in the ions involved. Unlike the action potentials in animal cells which normally involve Na^+/K^+ exchange, plant cells, such as *Nitella* (Hope and Walker, 1975) and *Acetabularia* (Gradmann et al., 1973), have been reported to show an enhanced Cl^--efflux during the depolarization phase of its action potential. A detailed study of action potentials has been made with the relatively larger cells of some algae (e.g. *Chara*, *Nitella* and *Acetabularia*). Findlay and Hope (1964a) showed that potential

changes in the vacuole of *Chara* during the action potential consist of two components; (a) a large fall from about -150 mV to -25 mV in the potential across the plasmalemma with recovery in about 3 seconds, and (b) a slow increase in the potential across the tonoplast from about 25 mV to 50 mV with a gradual recovery to the resting level after about 20 seconds. During this period, the resistance of the plasmalemma falls by a factor of about 40 while that of the tonoplast falls by a factor of about 3. On the basis of results obtained from voltage-clamping techniques, Findlay and Hope (1964b) concluded that the lowered electrical resistance of the plasmalemma reflected a large transitory increase in its anion permeability and that the resulting efflux of Cl^- from the cytoplasm caused the membrane to depolarize. The transient permeability to Cl^- and other anions is a function of the concentration of Ca^{2+} in the internal medium. Subsequent repolarization was the result of a loss of cation (principally K^+) by the cell. Similar mechanisms for the ionic basis of action potentials have been reported for *Nitella* (Mullins, 1962) and *Acetabularia* (Gradmann, 1970).

The basic requirement for the production of action potentials appears to be a momentary depolarization of the plasmalemma potential to a threshold level. Various types of stimuli may bring about this initial depolarization leading to an action potential in a charophyte cell; e.g. a sudden application of pressure to a region of the cell (Kishimoto, 1968), a sudden change of temperature (Harvey, 1942), or a pulse of electric current (Fujita and Mizuguchi, 1955). In *Acetabularia*, action potentials arise spontaneously or can be initiated by lowering the temperature (Gradmann, 1970).

Well-defined action potentials have been demonstrated in higher plants too and in some cases can propagate from cell to cell. Such propagating action potentials have been shown to occur in cells of plants showing rapid movements, e.g. *Mimosa* (Sibaoka, 1966), *Dionaea* (Sibaoka, 1966; Benolken and Jacobson, 1970) and *Drosera* (Williams and Pickard, 1972a, b; Williams and Spanswick, 1972). The action potentials in these plants, which arise due to mechanical stimulation, appear to control their movements. In *Dionaea* (Venus fly-trap) they are initiated in sensory hairs and propagate to the motor cells where they bring about trap closure. These action potentials have an amplitude of about 100 mV in the cells of the trap-lobes, a rise time of 0.1 to 0.2 s, a duration of 0.6-1.0 s, and a rate of propagation ranging from 60 to 170 mm. s^{-1}. In *Mimosa* the signals appear to be conducted by specialised cells. Certain parenchyma cells in the protoxylem and phloem which have larger membrane potentials than their neighbours, generate and conduct action potentials with a rise time of about 0.5 s, a total duration of several seconds and a velocity in the range of 20 to 30 mm. s^{-1}. The leaflets can be seen to fall in sequence as the action potential propagates along the rachis. In *Drosera* a mechanical disturbance at the head of one of the leaf tentacles produces a depolarization of adjacent cells in the stalk. Action potentials of duration 3 to 15 s are initiated if the depolarization reaches a threshold value and are propagated from cell to cell to the base of the tentacle with a velocity of about 5 mm. s^{-1} and control bending. There are many other excitable systems in the plants of several other families and work on these has been reviewed by Sibaoka (1969).

Although the above mentioned plants, which have adapted action potentials as a means of communication to mediate mechanical

movement, are among a minority in the plant kingdom, there are many non-motile plants which also show electrical fluctuations resembling action potentials. By taking proper precautions to eliminate noise and by using high amplification, trains of small, highly patterned voltage fluctuations have been recorded from the shoots of *Ipomoea, Xanthium, Pisum* (Pickard, 1972) and *Ficus elastica* (Karlsson, 1972). It is quite possible that this type of spontaneous electrical activity is a universal phenomenon in the plant kingdon. The significance of this activity, however, remains obscure; it might be merely an incidental accompaniment to a physiological process or processes, or may serve some useful but still unknown function.

Although no detailed study on the ionic basis of the action potentials in higher plants has been carried out (perhaps due to the technical difficulties), these phenomena are undoubtedly the result of rapid changes in membrane ionic conductance and permeability and may well be accompanied by other, less easily observed physiological effects.

One physiological phenomenon thought to be linked to electrical activity was phloem translocation, for example an electroosmotic theory of phloem transport was proposed by Fensom (1957) and Spanner (1958). The theory has been modified many times since its inception, the most recent being in the form of a restatement by Spanner (1979) in the light of new discoveries. The previous potassium pump has now been replaced by a proton excretion pump which provides the electroosmotic driving force. It was proposed that such a mechanism for phloem translocation might generate transient electrical activity superficially resembling action potentials. It should be possible to check for these potential changes by recording from fine metal electrodes in or near the vascular bundles. Also it would be

pertinent to investigate the possibility of any association between the spontaneous trains of electrical fluctuations, which have so far been overlooked, and phloem activity.

Not all action potentials occur spontaneously, a variety of treatments can evoke electrical fluctuations in non-motile as well as motile plants. There are reports in the literature of propagating, large electrical fluctuations in many plants, generated as a result of injury (cutting, breaking or burning), as in the case with animal nerve impulses where the number of action potentials induced depends on the severity of the stimulus.

Umrath (1959) reported travelling electrical fluctuations, induced as a result of injury, in wide variety of plants including *Cucumis*, *Cassia* and *Phaseolus*. Conduction velocities in these plants ranged from 1 to 15 mm. s^{-1}. In the 1960's, in a series of publications, Sinyukhin and coworkers reported electrical activity induced by various types of treatments. Gunar and Sinyukhin (1962), by placing electrodes on the vascular bundles, recorded one or more spreading "waves of excitation" induced by heat or 1 N KCl treatment of the roots of pumpkin, plantain and kidney bean plants. They proposed that the "waves of excitation" might carry information from one organ to another. The "waves of excitation" were later termed as "action currents" by Gunar and Sinyukhin (1963). They travelled at a rate of about 10 mm. s^{-1} in the conducting system of the stem and petiole and on reaching the leaves induced a change in the "metabolic potential" (this was measured from tissue areas where the main conducting bundles of the leaf were not located). They claimed that KCl-induced "action currents" resulted in a change in the "metabolic potential" of the leaf which was followed by an increase in the rate of photosynthesis and a drop in the rate of

respiration within a few minutes. However, a heat stimulus to a leaf, which also induced an "action current" and similar change in the "metabolic potential" in neighbouring leaves, resulted in a drop in their rate of photosynthesis and increased respiration. Stimulation of "action currents" by some other agents (IAA, GA and 2,4-D at a concentration of 10^{-2}M) again produced conflicting responses with regard to CO_2-exchange. More recently Van Sambeek and Pickard (1976a, b) have carried out a detailed investigation into heat-induced electrical activity. They claim that a chemical factor released as a result of heating the leaves depolarizes the cells and can be transported in the transpiration stream. It can give rise to a spreading variation potential (a change in potential which takes a long time to return to the base-line) throughout the shoot, which is often accompanied by action potentials. However, the nature of KCl-induced signals remains little understood inspite of the large number of reports by the Russian workers, e.g. it is not known whether the KCl-induced signals are due to its osmotic effect on turgor or some other mechanism. If the KCl-induced signals in roots are a result of osmotic stress then it is possible that plant roots can produce these signals in nature (during drought). The significance of these signals might be in communication, provided they can travel to various parts of the plant (e.g. a message to the leaf to close the stomata during drought condition). It would clearly be interesting to investigate the effects of other types of stresses on the root, e.g. oxygen deficiency during waterlogging of the roots etc. Thus a study of the basic mechanism of the signals produced by the roots might provide some important clues to their possible significance.

The evidence presented above is more than enough to arouse one's interest into the fascinating field of electrical activity in plants and to stimulate investigation into its many aspects, such as the phytochrome-mediated electrical phenomenon, the spontaneous trains of action potentials and the little understood KCl-induced signals in the roots.

2. MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 Phytochrome-mediated Bioelectric Potentials

2.1.1 Plant Material

Wheat (*Triticum vulgare*, var. Sicco) seeds were grown in vermiculite in 1 mM $CaCl_2$ solution. The seeds were placed in an inverted lid of a plastic sandwich box (22 x 11 x 8 cm, from Stewart Plastics Ltd.) and then covered with a shallow layer of vermiculite. After soaking the seeds and vermiculite with 100 ml of 1 mM $CaCl_2$ solution the lid was covered with the bottom of the sandwich box and placed in a dark room at 25°C. After 4 days the coleoptiles of the seedlings had grown to an average length of 4.5 cm. The seedlings growing straight were selected, the vermiculite washed off their roots and they were then placed with their roots and endosperm submerged in 10 mM KCl for about 2 hours to equilibrate before use. The necessary manipulation of the seedlings was carried out under a dim green safelight. The exposure to the green light was kept to minimum (2-3 minutes).

2.1.2 Lights

The safelight was a 60 W tungsten bulb behind a Kodak green safelight filter placed about 85 cm above the working area and was used only when illumination was required.

Red (R) light was obtained from a 15 W fluorescent tube wrapped in a layer of red cinemoid (No. 14, supplied by Rank Strand Electric, Brentford, Middlesex, TW8 9HR). Each end of the tube was covered with 17 cm of black tape leaving a small window for the red light to emerge. The energy of the light reaching the coleoptile

was 8 $\mu W.cm^{-2}$.

The far red (FR) light source was a 150W projector with a 732 nm Balzers interference filter in the slide carrier. The whole projector assembly was placed in a box to eliminate stray light. The FR source was 25 cm away from the coleoptile and delivered an energy of 7 $\mu W.cm^{-2}$.

The irradiance of the light sources were measured with a spectroradiometer (ISCO, Lincoln, Nebr. 68507).

2.1.3 Electrical Measuring Techniques

Measurements were made at 20°C, under high humidity. Mercury-calomel electrodes were used for surface potential measurements with 10 mM KCl solution as a contact medium between the electrode and the surface of the seedling. The reference electrode was in contact with the 10 mM KCl solution surrounding the roots and the endosperm of the seedling. The recording electrode was in contact with the apical 3-4 mm of the coleoptile. The two electrodes were connected to a high input impedance amplifier, with the reference being connected to ground. The output of the amplifier was fed into a chart recorder (Servoscribe 1S, Smiths Industries Ltd.). The apparatus used is shown in Figure 2.1.

The input resistance of the amplifier was in excess of 10^{12}-ohms which was sufficiently high for the measurement of the surface potential of the seedling. Figure 2.2 shows the circuit diagram of the high input impedance amplifier used.

The whole of the coleoptile was normally exposed to red or far red light except when determining the area of perception in which case part of the coleoptile was shielded from the light. The roots, endosperm and electrodes were always shielded from the lights.

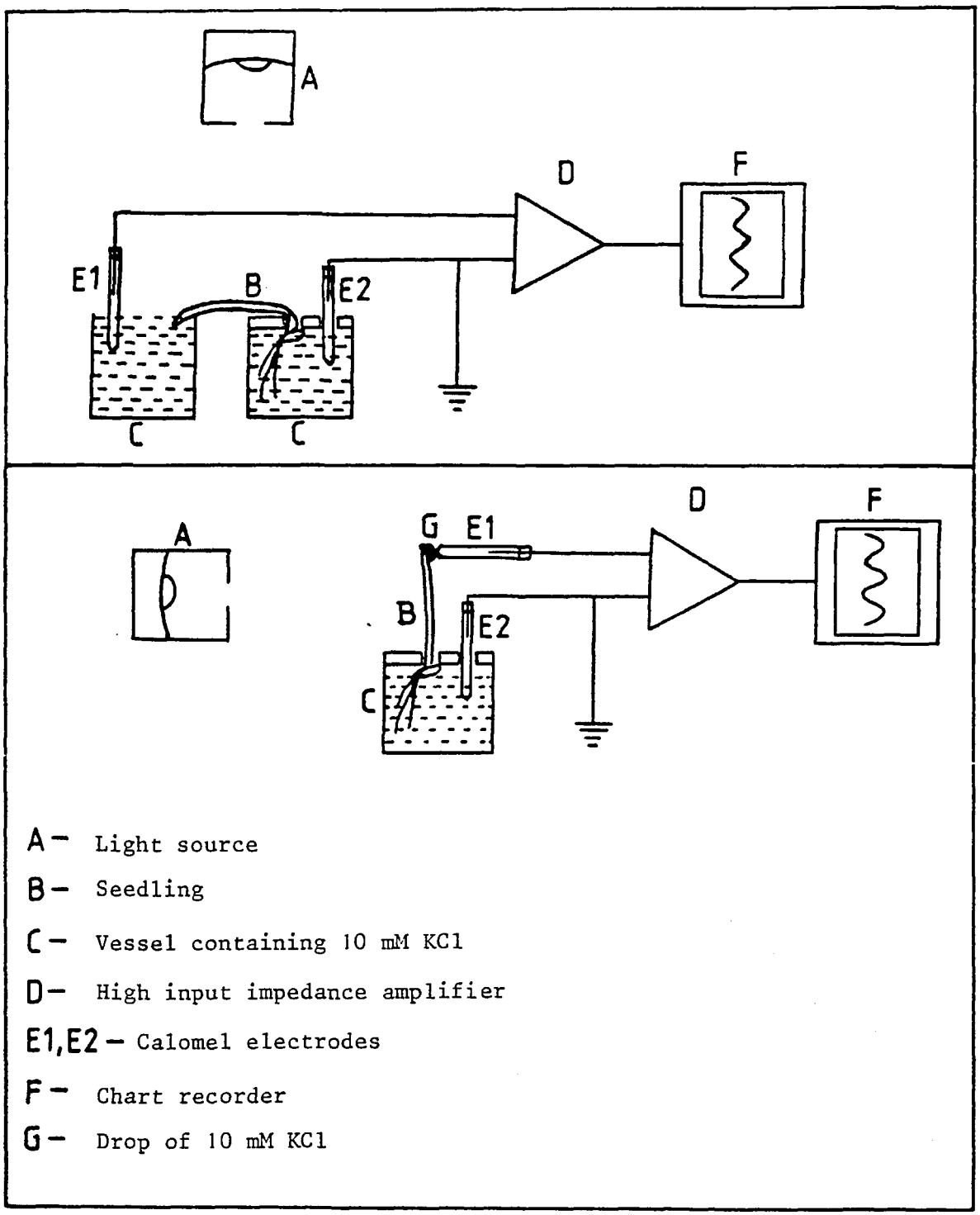


Fig. 2.1 Diagram of two alternative experimental arrangements used to measure phytochrome-mediated potential changes. Both arrangements gave similar results.

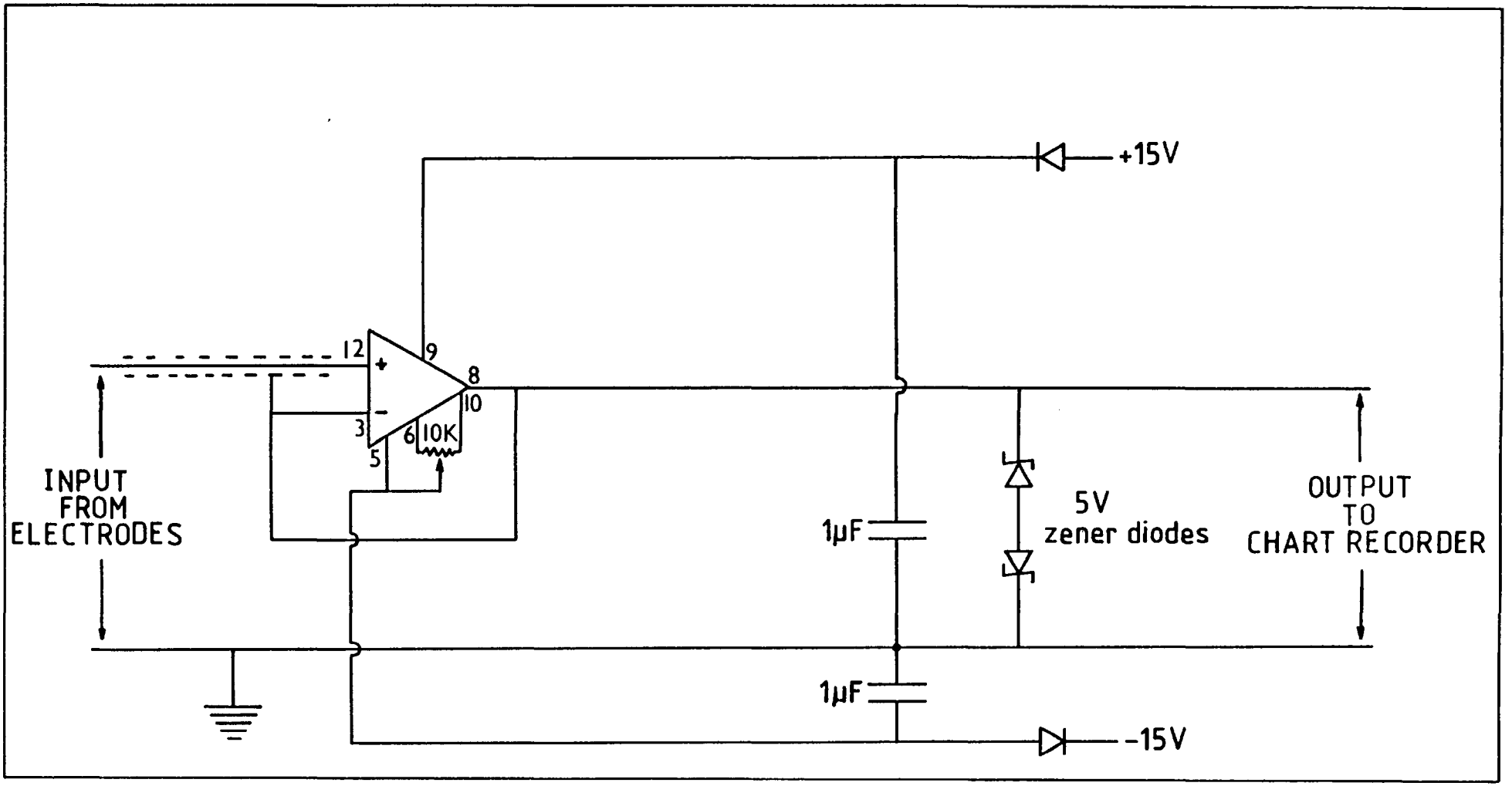


Fig. 2.2 Circuit diagram of the high input impedance amplifier used for the measurement of phytochrome-mediated potential changes. The I C is a F.E.T. input operational amplifier type 305-945 from R S Components Ltd., London.

2.2 Bioelectrical Activity in Plants Grown in Light

2.2.1 Spontaneous Electrical Activity

2.2.1.1 Plants

Seeds of marrow (*Cucurbita pepo*, var. long green striped) and pumpkin (*Cucurbita maxima*, var. hundredweight) were obtained from Suttons Seeds Ltd., Torquay and Schmidt Litho. Co., S.F., U.S.A., respectively. The seeds, which had been imbibed for 8 hours, were planted in John Innes potting compost in clay pots and grown in a greenhouse. Daylight was supplemented by a bank of 8 white fluorescent tubes giving 19 $W.m^{-2}$ for an 18-hour photoperiod. The air temperature in the greenhouse was maintained at $25^{o}C \pm 3$. 3-4 week old healthy plants were used for the experiments.

2.2.1.2 Recording Conditions

All the recordings were carried out at $25^{o}C$ under a fluorescent light with an energy of 0.6 $W.m^{-2}$ at plant level.

2.2.1.3 Electrodes

2.2.1.3.1 Tungsten Electrodes

These were made from tungsten wires of 0.2 mm diameter obtained from Clark Electromedical Instruments, Pangbourne, Reading. A 1.5 cm long piece of this wire was inserted halfway into a stainless steel hypodermic needle and crimped in place. The other end of the tungsten wire was sharpened, using an electrolytic process, to a tip diameter of a few microns. Saturated KNO_2 solution served as the

electrolyte. The electrodes consisted of a carbon rod and the tungsten wire to be sharpened, 4 volts AC being applied between the two. The carbon rod was kept immersed in the electrolyte continuously and the tip of tungsten wire to be tapered was dipped into the electrolyte, moving it around at the same time with fingers, and taken out immediately. This process was repeated many times and the progress of the etching was periodically checked under the microscope until the desired sharpness achieved. The electrode was washed with detergent and then with acetone. The electrode was then insulated, except for the extreme tip, with epoxylite resin to give a tip impedance of 5-12 Megaohms. To do this the electrode was suspended on a string with its tip pointing downwards, dipping into thickened epoxylite resin. The string was wound around the axis of a small, slow electric motor which, when switched on, lifted the electrode slowly out of the resin at about 3 mm/min. This process was repeated 3 times and then the electrode left to dry at the room temperature. The electrodes prepared in this way were placed vertically, with their tips pointing upwards, in an oven for 30 minutes at 98°C and then for another 30 minutes at 125°C. The heating process polymerized the resin and opened up the extreme tip.

Each electrode was tested microscopically to check for insulation integrity. The test was carried out by making the insulated part of the tungsten wire as one of the electrode placed in a 0.9% NaCl electrolyte. A platinum wire served as the other electrode. An increasing DC voltage of up to 3 volts was applied between the electrodes and the insulated tip was observed under the microscope. The position of uninsulated part was indicated by bubble formation on it. Electrodes with one small opening at the tip were selected for experimental use.

2.2.1.3.2 Silver/Silver Chloride (Ag/AgCl) Electrodes

4 cm long pieces of 1 mm diameter silver wires were chloridized by an electrolytic process. These wires, immersed in 1 mM KCl electrolyte solution, served as anodes. Another silver wire, placed few centimetres away, served as a cathode. 1 volt DC was applied between the electrodes for 10 hours. This process was carried out in dark as silver chloride is reduced back to silver in the light. Freshly chloridized silver wires were inserted into glass pipettes containing 3 M KCl in 2% agar (Oxoid Ltd., London S.E.1). Through the tip extended a short length of cotton thread soaked in the saline agar.

Preliminary recordings, of the small naturally occurring or the large stimulated action potentials, showed that they could be recorded equally well using either type of electrode. Therefore, to eliminate the problems of drying out of saline wicks which occur when Ag/AgCl electrodes were used, tungsten electrodes, inserted into tissue, were employed in most cases when recording for long periods. The tungsten electrodes, although very fine and inserted inside the tissue, gave signals which were similar to those from external Ag/AgCl electrodes.

2.2.1.4 Contacts

The tungsten electrodes were fitted onto insulated holders which could then be fixed onto micromanipulators (Prior, England). The part of the plant used for electrical recording was secured firmly into position by fastening it against a fixed platform with adhesive tape. The tungsten electrode was then inserted in the tissue to a depth of 0.5-1 mm using the micromanipulator. A stainless steel rod was put in the pot to serve as a reference electrode

which was connected to ground at the amplifier.

The Ag/AgCl electrodes were positioned in such a way that their salt wicks were touching the surface of the plant. A drop of 10 mM KCl served as a contact between the electrode and the plant surface. Small drops of distilled water were added to the wick from time to time to prevent its drying and hence poor contact. The plant was earthed at the pot with a chloridized silver wire.

To avoid the danger of mechanical vibrations and electrical noise most of the recordings were carried out in a Faraday cage in an isolated room.

2.2.1.5 Recording Equipment

The recording electrode was connected to a high input impedance amplifier with a gain of X10. To reject the DC drift during recording which is inevitable when metal electrodes are used over long time intervals, and in order to be able to use higher sensitivities of the chart recorder, the output of the amplifier was connected to a high pass filter (time constant - 20s and gain X10). The output from this high pass filter was then connected to a type 28000 dual channel chart recorder from Bryans Southern Instruments Ltd. Two identical amplifiers were used when recording at two different points on the plant and the activities recorded separately on each channel. The apparatus used is shown in Figure 2.3. The circuit diagram of the high input impedance amplifier coupled with the high pass filter is shown in Figure 2.4. The total gain of the circuit is X100. All the components to build the amplifiers, filter and power supply were obtained from R S Components Ltd., London.

The high pass filter was not used for every recording. Recordings made without the high pass filter are marked D.C., and

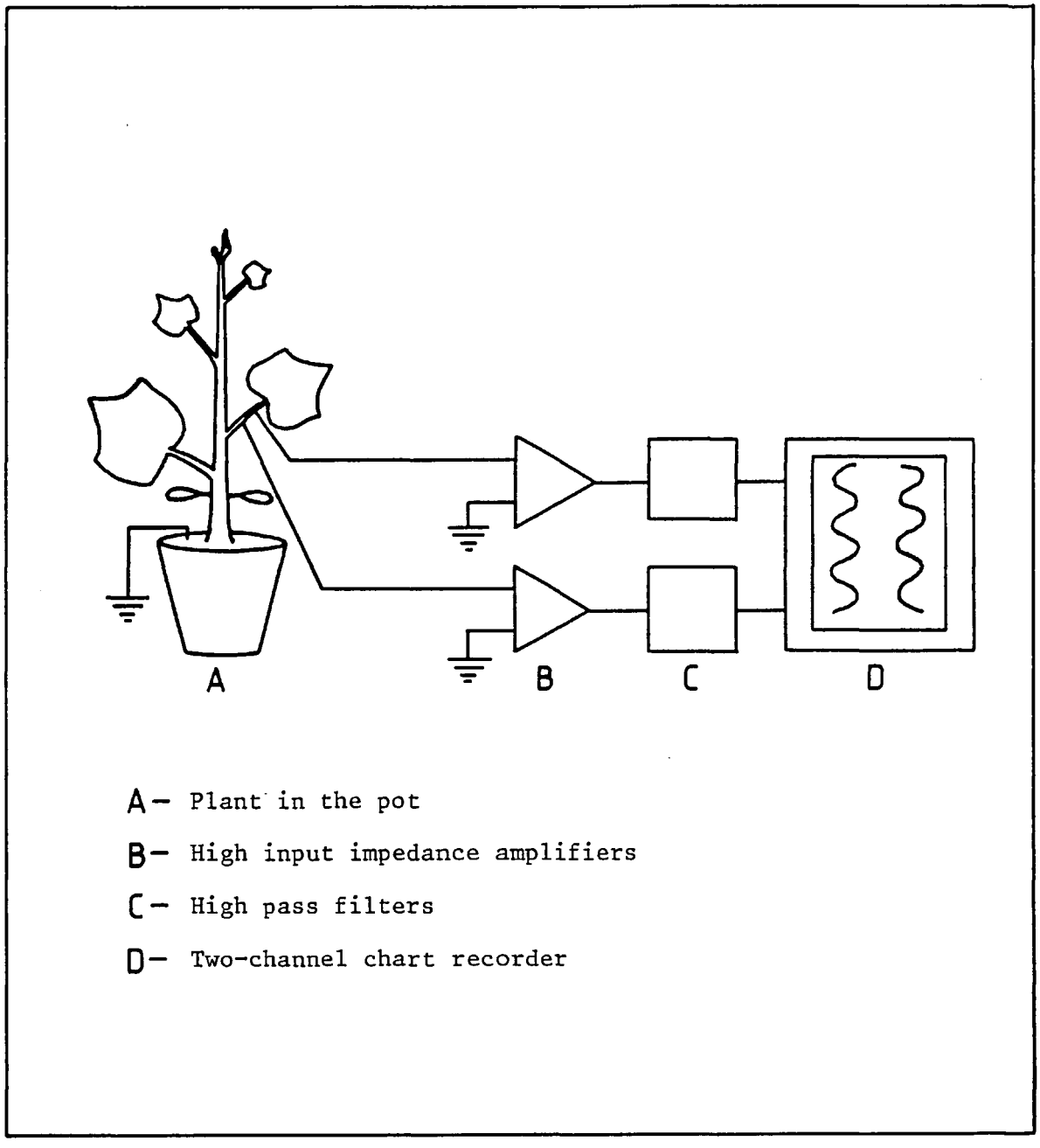


Fig. 2.3 Schematic diagram of the apparatus used for measurement of electrical activity in the light-grown plants.

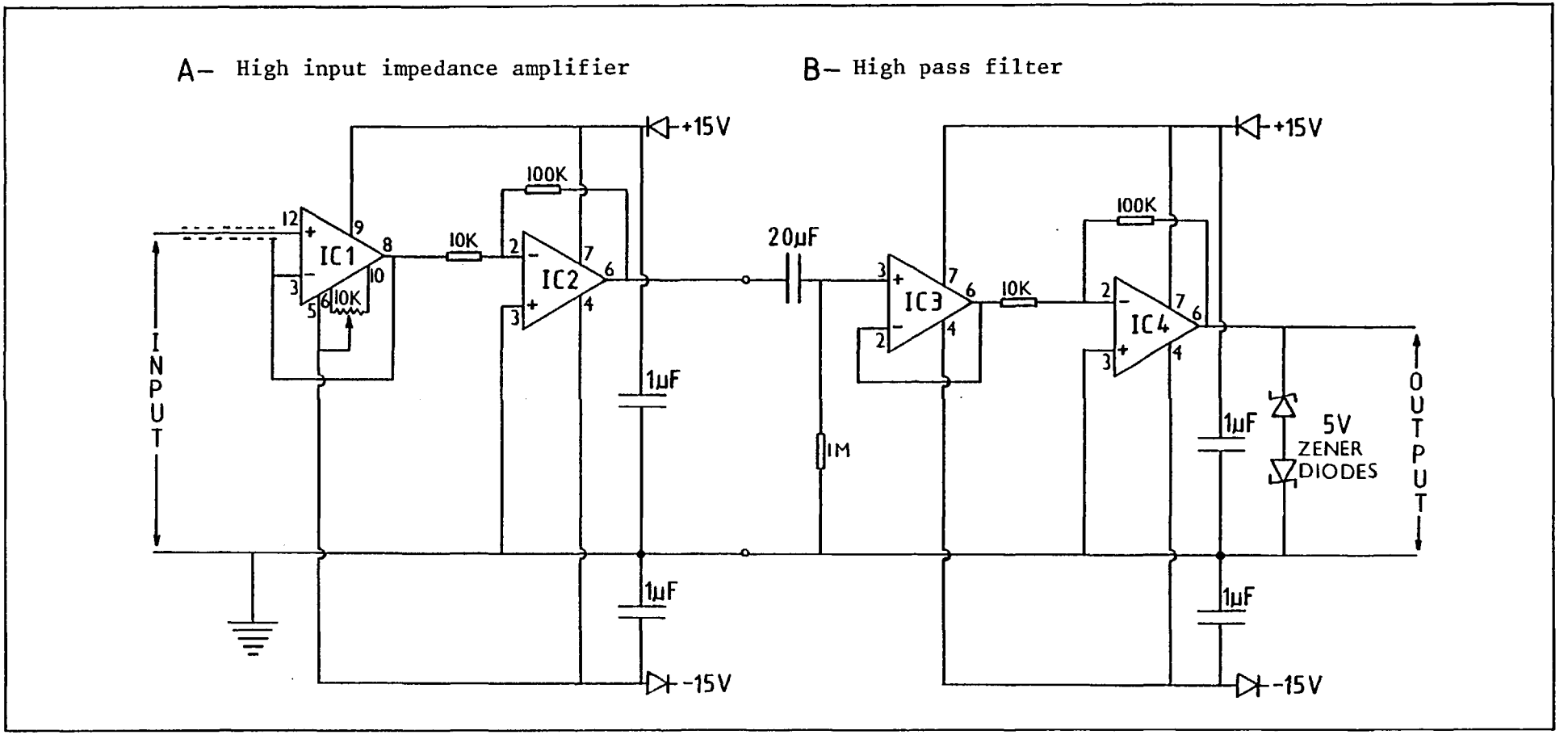


Fig. 2.4 Circuit diagram of (A) high input impedance amplifier, gain x 10 and (B) high pass filter, gain x 10, time constant 20 seconds. I C 1 is F.E.T. input operational amplifier (type 305-945) and ICs 2, 3 and 4 are 741 operational amplifiers (type 305-311) obtained from R S Components Ltd., London.

the ones with the filter in the circuit are marked A.C. since it makes the unit insensitive to D.C. (and also to A.C. signals which are appreciably longer than the 20 second time constant).

2.2.1.6 Electrical Shielding

All the experiments were conducted with the plant, electrodes and amplifiers contained in a Faraday screened cage which was connected to ground. This way most of the interference due to stray electrical fields, which are sometimes produced because of the presence of the investigator, was eliminated. It was checked by waving an ungrounded hand near the cage. Sometimes switching on or off of some heavy electrical equipment in the adjoining room caused sharp, negative-going spikes in the recording. However, such signals could be easily identified because of their very short duration and their occurrence usually coincided with an audible snap of relays in the equipment. Mechanical disturbances, which occurred rarely also produced sharp, negative-going spikes on the chart which again was easily recognisable because of their very short duration and the sound which accompanied them.

The mains supply, to all the equipment used for recording, was filtered through a mains suppression filter (type 238-441, obtained from R S Components Ltd., London) to eliminate mainsborne interference as far as possible.

2.2.1.7 Testing

The recording equipment was checked from time to time and specially when an interesting activity was occurring to make sure that it was of plant origin. This was done by connecting a dummy plant in the form of 10 M resistor in place of the electrodes to one

of the recording channels and recording the plant activity on the other.

2.2.1.8 Figures

The figures showing the electrical activity are tracings of the original recordings.

2.2.2 Stimulated Electrical Activity

Most of the recording conditions, equipment and techniques were similar to those previously described for spontaneous electrical activity. Marrow and pumpkin plants were used for these experiments. Following are the methods of stimulation.

2.2.2.1 Heat Stimulus

A part of the leaf blade was burned by passing the flame of a lighted match back and forth 10-30 mm below it for 5 seconds. The activity was recorded by two electrodes usually placed some distance apart on the petiole of the same leaf. In some cases one electrode was placed on the stimulated leaf petiole and another on a different petiole or on the stem to check acropetal or basipetal propogation of the signal.

Heat-induced signals were produced in roots by replacing the nutrient medium around the roots with a similar medium at 90°C. In such cases the reference electrode was placed in the nutrient medium and recording electrodes were on the aerial part of the plant.

2.2.2.2 Chemical Stimulus at the Aerial Parts of the Plant

A range of IAA (10^{-8}-10^{-4} M), 2,4-D (5.10^{-6}-5.10^{-5} M),

kinetin (5.10^{-6}-5.10^{-5} M), acetylcholine chloride (0.3 - 10 mM) and abscisic acid (10^{-4}-10^{-3} M), concentrations were applied to either a leaf or its petiole near the electrode.

2.2.2.3 Chemical Stimulus at the Roots

The plants were grown in soil. The soil from 3-4 week old plant was washed away and the plant was kept with its roots in an aerated nutrient medium (KCl, 10 mM; $Ca(NO_3)_2$, 10 mM; NaH_2PO_4, 9.05 mM; Na_2HPO_4, 0.48 mM and $MgSO_4$, 2.5 mM; pH 5.3) for 2 days in the greenhouse before use. The recording apparatus consisted of the plant with its roots in the nutrient medium, the recording electrodes at two points on the aerial part of the plant and the reference electrode in contact with the medium surrounding the roots. The stimulus was provided by replacing the nutrient medium with a solution of the desired concentration of the following chemicals in the medium; KCl (0.1-1 M), NaCl (0.2-0.5 M), $CaCl_2$ (0.2-0.5 M), IAA ($10^{-12}$$10^{-4}$ M), 2,4-D (5.10^{-6}-10^{-4} M), ascorbic acid (10-100 mM), kinetin (5.10^{-6}-5.10^{-5} M), acetylcholine chloride (10^{-5}-5.10^{-3} M), abscisic acid (10^{-4}-5.10^{-4} M) and KCN (3-10 mM).

2.2.2.4 Osmotica and Removal of Water Stress

A stimulus was provided to the roots by creating or removing a high osmoticum around them. This was done by putting the roots in 0.1-0.8 M mannitol and then replacing it with nutrient medium. Another method employed was the watering of wilted plants.

2.2.2.5 Anaerobiosis

To examine the effect of anaerobiosis on root electrical activity, nitrogen was passed through the bathing medium.

2.2.2.6 Light

Part of the plant or whole plant was either kept in dark or in light (30 $W.m^{-2}$) and its electrical activity recorded.

2.3 Transmembrane Potential Measurements

2.3.1 Plant Material

Marrow seeds, after 4 hours of imbibition in a beaker of water, were grown on filter paper in distilled water inside plastic sandwich boxes for 3 days in dark at 25°C. When the roots had grown to about 2 cm, the apical 1 cm of the root was excised and incubated in dark in aerated bathing medium (KCl, 10 mM; $Ca(NO_3)_2$, 10 mM; NaH_2PO_4, 9.05 mM; $Na_2H\ PO_4$, 0.48 mM; $MgSO_4$, 2.5 mM; pH 5.3) for half an hour before use.

2.3.2 Microelectrodes and Instruments

Glass microelectrodes were made from 2 mm (outer diameter) glass capillaries containing a microfibre (supplied by Clark Electromedical Instruments, Pangbourne, Reading, England). These capillaries were pulled into micropipettes using a microelectrode puller (C.F. Palmer (London) Ltd.). The micropipette was filled with 3 M KCl, a chloridized silver wire was inserted and connected to the high input impedance amplifier (Figure 2.4A). Acceptable electrodes had a tip diameter of less than 1 μm and a tip resistance of 10-20 megaohms. A reference electrode was made from 2 mm (outer diameter) glass tubing, filled with 3 M KCl in 2% agar, and connected via a Ag/AgCl wire to the ground at the amplifier. A micromanipulator (de FONBRUNE No. 72, CH. BEAUDOUIN CONSTR. PARIS) was used for the insertion of the microelectrode into the cells.

2.3.3 Measurement of Membrane Potential in Root Cells

The root segments were mounted horizontally in an aerated bathing medium in a perspex chamber at 25°C. The microelectrode was inserted perpendicularly to the main axis in a region between 3 to 5 mm from the root tip and to a depth corresponding to the 3rd to 5th layer of cortical cells. It was not possible to see the position of the electrode tip inside the root tissue but its presence inside a cell was indicated by a sudden fall in the potential reading on the recorder on penetration. When the membrane potential had stabilized sufficiently, the chemicals (IAA, KCl, mannitol, ascorbic acid and KCN) were added to obtain the desired final concentration. When required an anaerobic environment was created by passing nitrogen rather than air through the bathing medium. The recordings were made on a chart recorder (Bryans 28000).

2.4 Determination of the Rate of Photosynthesis and Respiration

Pumpkin plants were used for these experiments. An apparatus was assembled to measure the rate of photosynthesis or respiration of a leaf and the electrical activity from the same leaf petiole simultaneously (Figure 2.5).

The leaf of a plant used for measurement was suspended in a flat transparent plastic chamber which was sealed with lanolin paste. Air from a cylinder (British Oxygen Company) was passed through the chamber, the flow being first regulated by a flostat and measured on a flowmeter (both supplied by G.A. Platon Ltd.). During respiratory measurements an additional sodalime tower was connected between the flowmeter and the leaf chamber to absorb all the CO_2 from the air. The leaf was kept in dark during respiratory measurements and was illuminated with light (26 $W.m^{-2}$) during photosynthetic

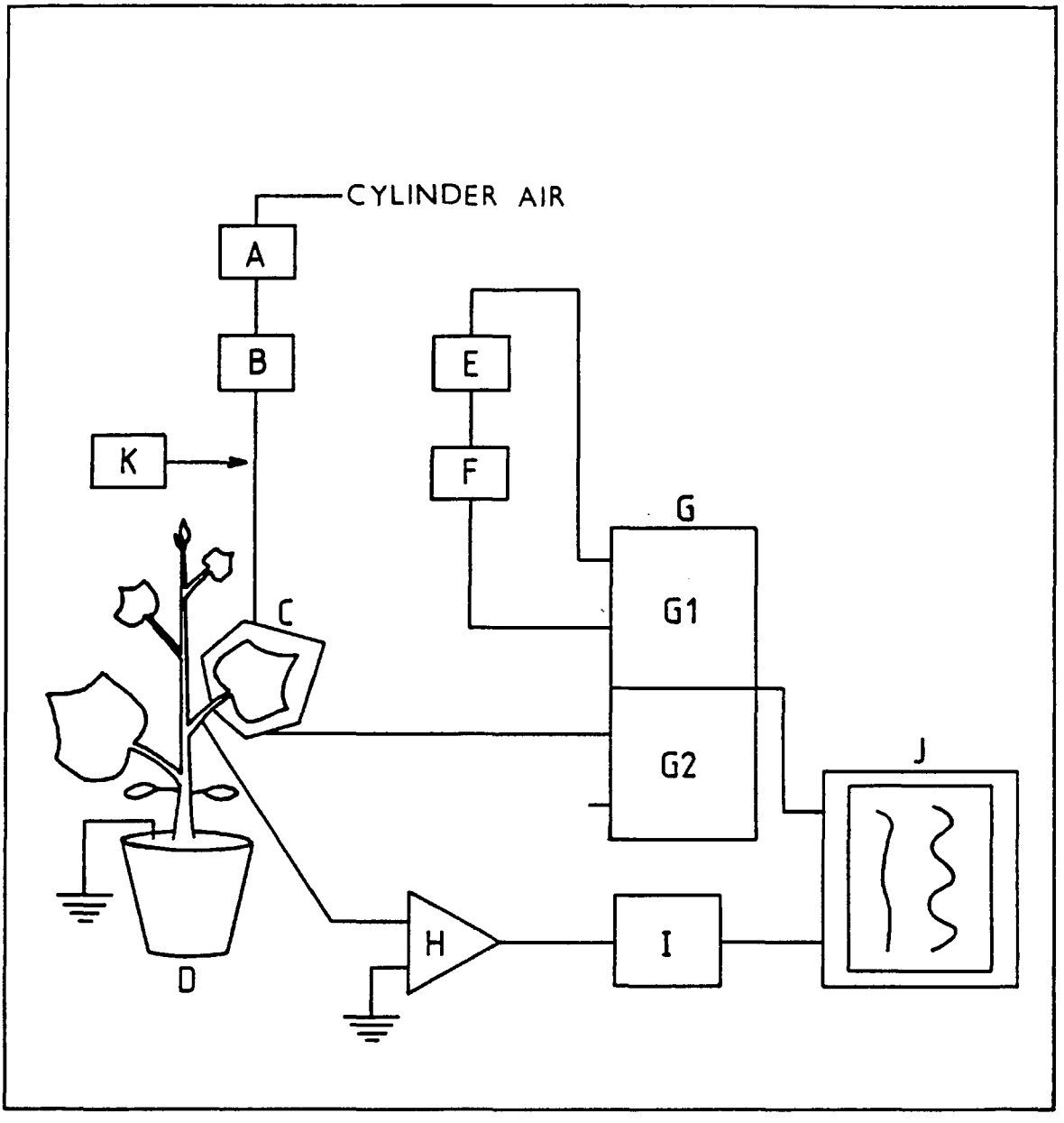


Fig. 2.5 Block diagrams of the apparatus used for recording electropotentials and CO_2 exchange in *Cucurbita* plants. (A) Flostat, (B) Flowmeter, (C) Leaf chamber, (D) Plant in the pot, (E) Air pump, (F) Soda lime tower, (G) Infrared gas analyser, (G1) Reference cell, (G2) Analysis cell, (H) High input impedance amplifier, (I) High pass filter, (J) Two-channel chart recorder, (K) Soda lime tower used only for respiratory measurements.

measurements. The air coming out of the leaf chamber was fed into the analysing cell of an infrared gas analyser (Uras 2, Hartmann and Braun). CO_2 free air was passed through the reference cell of the analyser. The electrical output of the analyser was recorded on one of the channels of a Bryans 28000 chart recorder. The gas analyser was checked for stability from time to time and the calibration checked between experiments. The leaf was equilibrated in the chamber for at least an hour before gas exchange measurements began.

The values of CO_2 concentration in the air stream from the leaf chamber were converted to rates of uptake or release by multiplying by the flow rate.

A tungsten electrode inserted in the petiole was used to pick up the electrical activity. The recording techniques were the same as described in section 2.2.2 of this chapter. KCl treatment of the roots and the heating of an adjacent leaf were the only stimuli used. The signals were recorded simultaneously on the second channel of the chart recorder.

3. RESULTS

3 RESULTS

3.1 Phytochrome-mediated Bioelectric Potential Changes in Wheat Seedlings

3.1.1 Preliminary Experiments

Preliminary experiments were carried out using wheat and barley coleoptiles and mung bean epicotyls. Only wheat showed any consistent red-far red reversible changes in the potential of the tip of coleoptile relative to the base and therefore, it was used for all the remaining experiments. A rise in the potential at the tip, relative to the base, occurred after red light and a fall in potential after far red light treatment. This cycle could be repeated many times. The recording calomel electrode placed in contact with apical 3-4 mm of the coleoptile gave maximum response and therefore this position was chosen as a standard one. The reference calomel electrode was in contact with the bathing medium surrounding the roots and the endosperm.

3.1.2 Potential Changes

In order to demonstrate that the observed potential changes were phytochrome-mediated, the coleoptile was subjected to a series of red and far red light treatments. Figure 3.1 shows a recording of electrical changes produced in response to a series of 6 cycles of red and far red treatments. Red light induced a rapid rise and far red light induced a rapid fall in the potential. The change in potential started after 5-10 seconds of the treatment. Initially, the magnitude of the response to red or far red light was rather small (2-4 mV) but later it increased (5-7 mV). Many seedlings were used

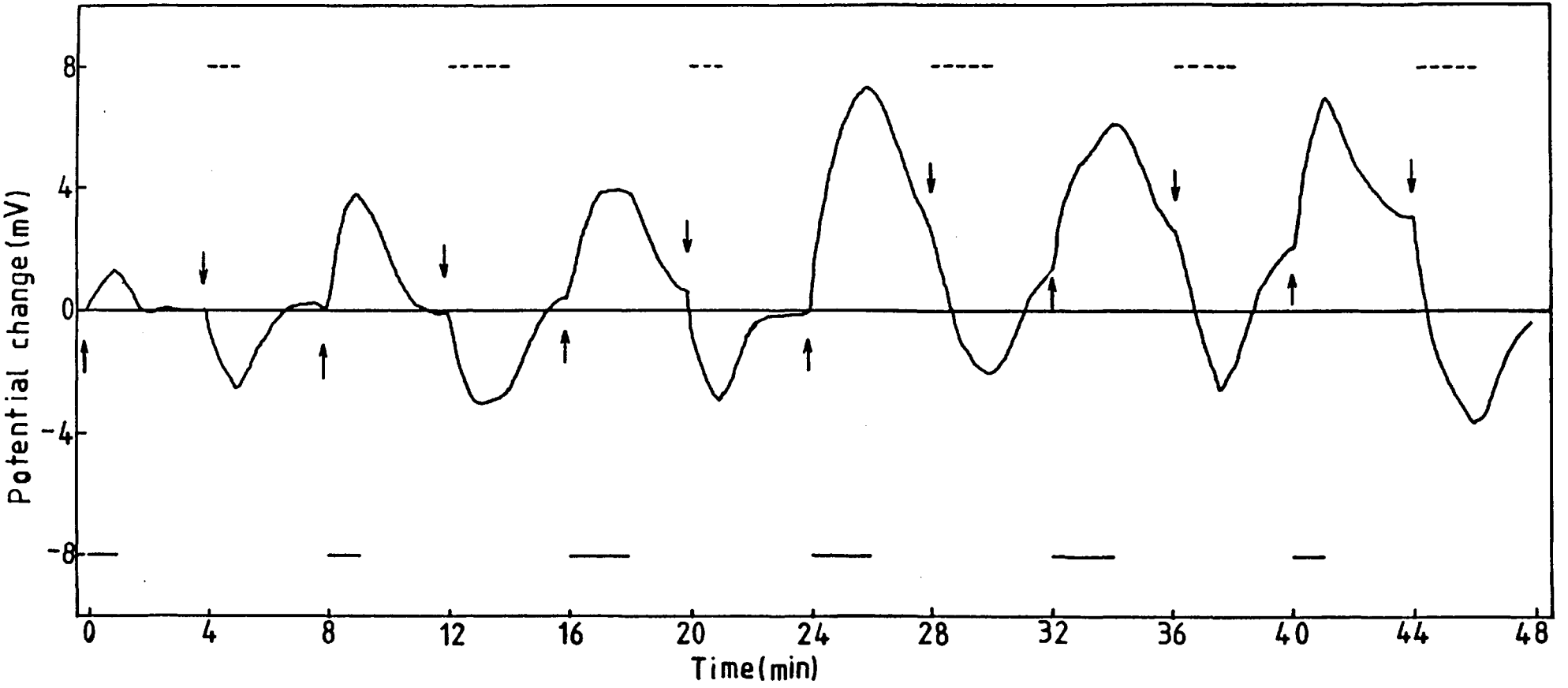


Fig. 3.1 A typical recording of electrical responses of a wheat coleoptile to a series of R (————) and FR (-------) treatments. The start of each treatment is indicated by an arrow, and its duration is indicated by the length of the solid or dotted bar. Successive R and FR treatments were separated by a dark period. Zero on the graph is the initial steady potential in the dark.

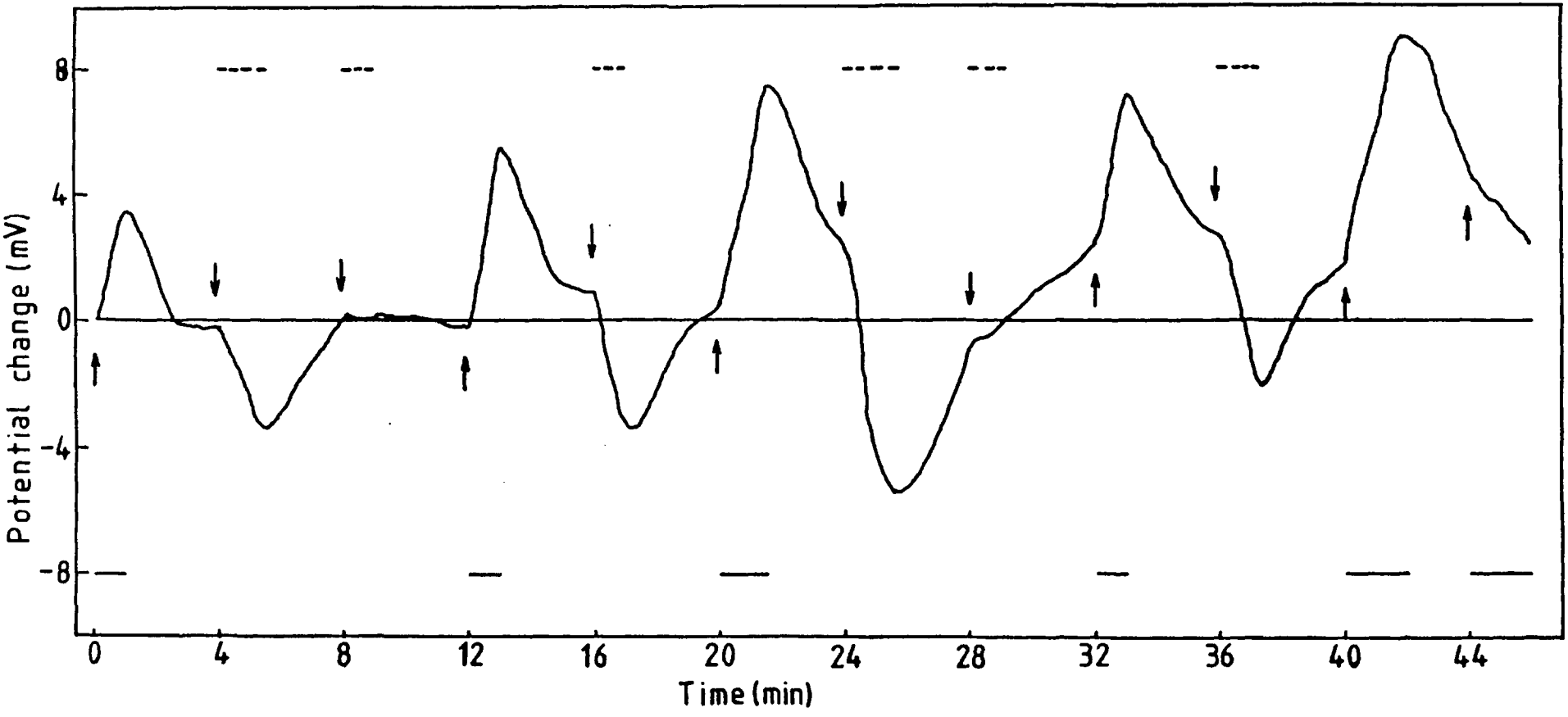


Fig. 3.2 A recording showing the effect of two successive R (———) and two successive FR (-----) treatments on the coleoptile potential. The start of each treatment is indicated by an arrow, and its duration is indicated by the length of the solid or dotted bar. Successive R and FR treatments were separated by a dark period. Zero on the graph is the initial steady potential in the dark.

for this type of experiment and most responded in a similar way to red and far red light treatments.

A typical result of another series of experiments is shown in Figure 3.2. The second red light treatment without the intervening far red did not induce a further positive change in potential, indicating that the first dose of 2 minutes was saturating. The first red light exposure given to an etiolated coleoptile always induced a positive change in potential. There is also some indication that the magnitude of the negative response to far red depends on the size of the positive response from the preceding red treatment. Again a second far red exposure without an intervening red treatment was ineffective.

3.1.3 Area of Perception

Experiments were conducted in order to locate the area of stimulus perception on the coleoptile. The desired part of the coleoptile was exposed to the light while the rest was shielded with a black opaque plastic tube. Figure 3.3 clearly shows that all parts of the coleoptile were equally perceptive to the stimulus. The apical or basal part of the coleoptile when exposed to red and far red, produced the same size of response as when the whole of the coleoptile was exposed. It seems as though the mechanism of transmission of stimulus, responsible for potential changes at the tip, is a rapid one, possibly electrical.

3.1.4 The Non-participation of the Roots

Another series of experiments was conducted in order to find out the importance of roots for the electrical phenomenon. The apical half of the roots or the whole of the root system and endosperm

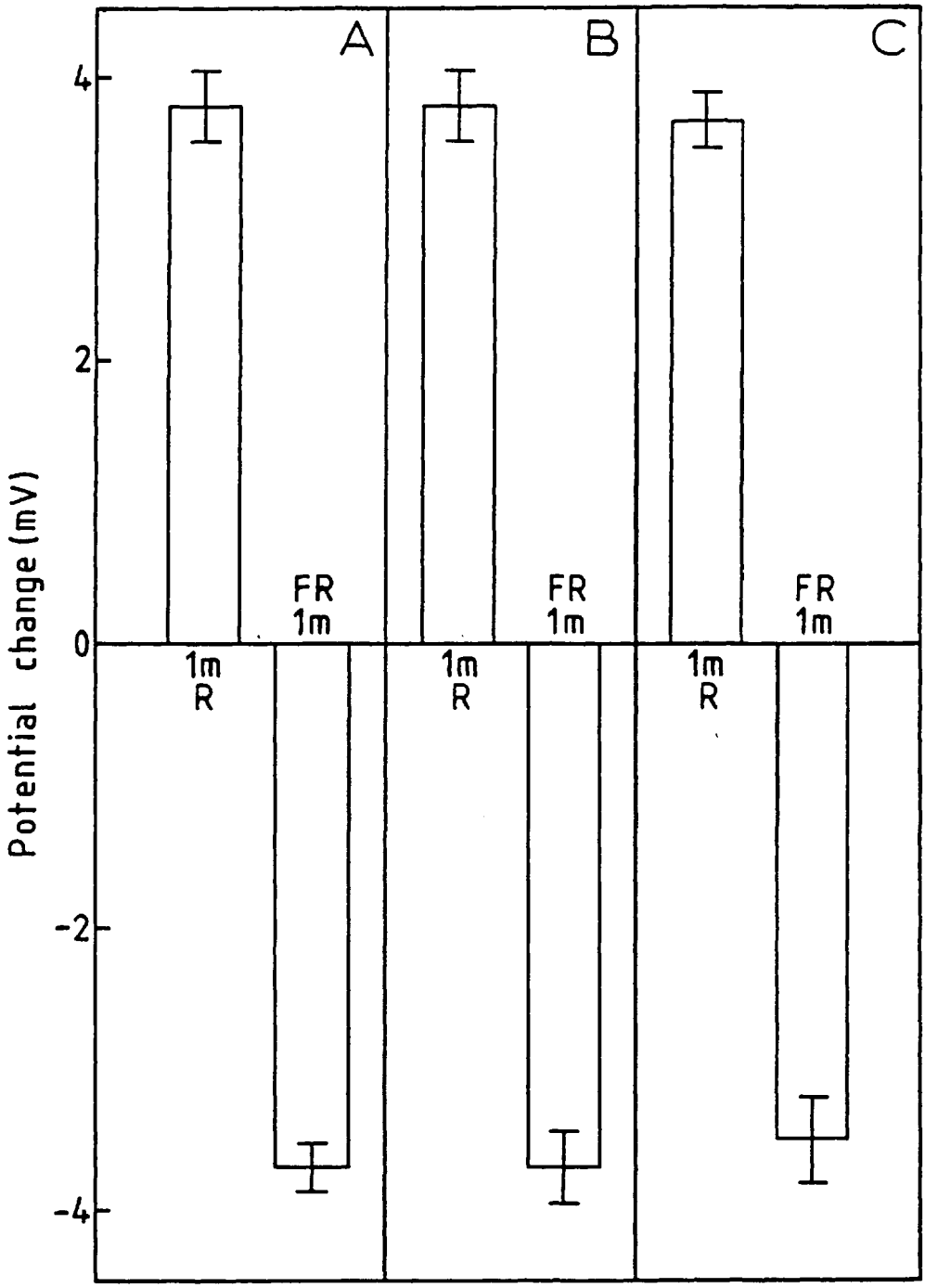


Fig. 3.3 Average potential changes following alternate 1 minute R and 1 minute FR treatments; when (A) the whole coleoptiles exposed, (B) the apical 1 cm of the coleoptiles exposed and (C) the basal 1 cm of the coleoptiles exposed. The same coleoptile was used for each treatment. The results are means ± SE (n = 11-13).

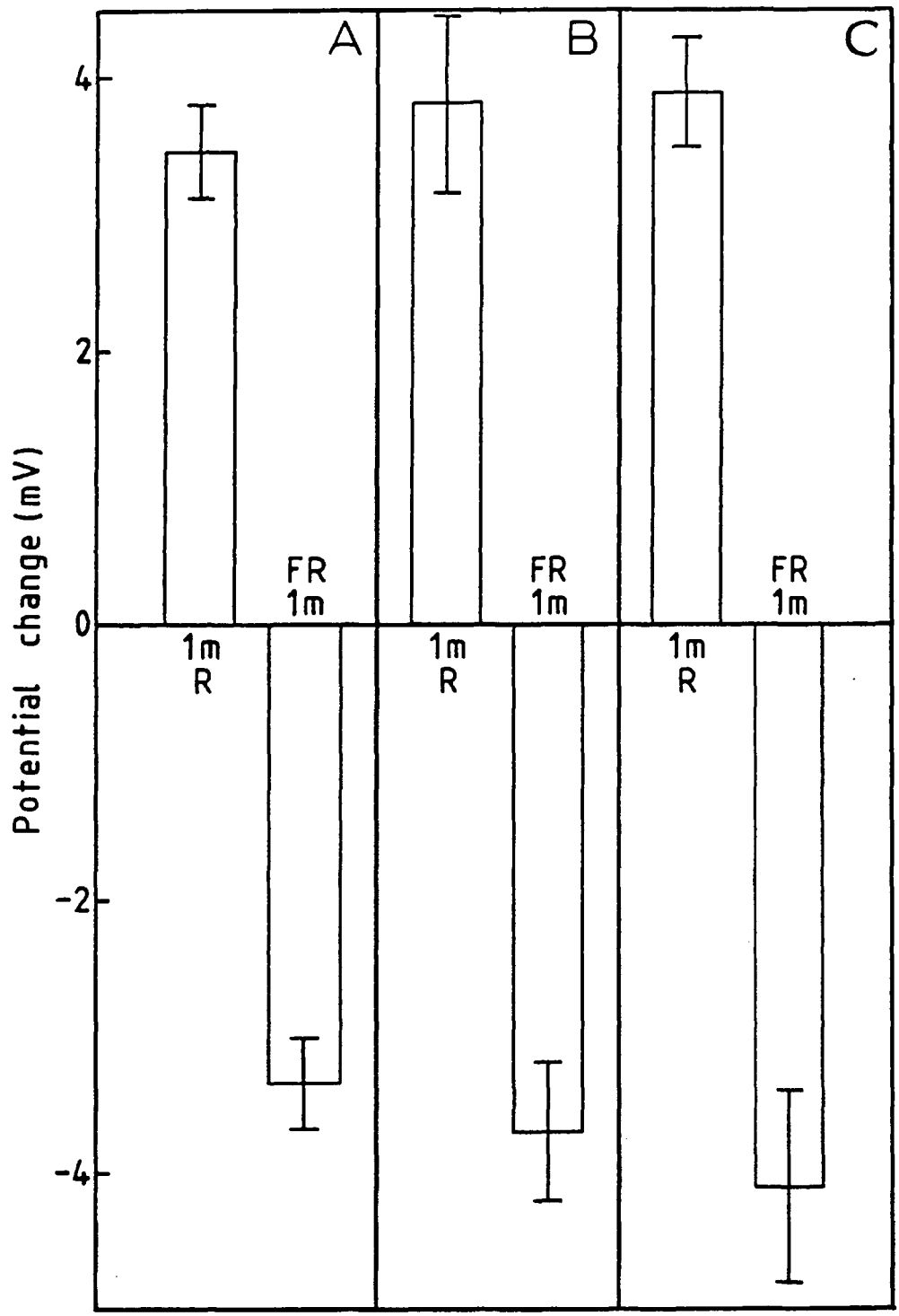


Fig. 3.4 Average potential changes following alternate 1 minute R and 1 minute FR treatments; (A) when intact seedlings were used, (B) after the root apices were cut off and (C) after the roots and endosperm were completely removed. The results are means ± SE (n = 5).

were cut off an hour before the first red light treatment. Figure 3.4 demonstrates that the absence of roots had no effect whatsoever on the phytochrome mediated potential changes at the coleoptile tip. It seems that the potential changes occur entirely between the tip and base of the coleoptile and the roots make no direct contribution.

A few similar experiments using isolated roots showed no phytochrome-mediated potential changes between their extremeties.

3.1.5 Responses to Red and Far Red by Decapitated Coleoptiles

The possibility of a relationship between the observed electrical changes and auxin was investigated using decapitated coleoptiles. The top 3 mm of the coleoptile was removed and measurements were made with the recording electrode in contact with the top 2 mm of the decapitated end. The coleoptiles did not lose their ability to respond to red and far red immediately after the decapitation (Figure 3.5). The maximum phytochrome-mediated response was observed for up to 25 minutes after decapitation, the response then declined and after an hour of decapitation the red-far red treatment had no effect (Figure 3.6B). It was thought that the loss of the IAA source, with the tip removed, was responsible for this and therefore, further experiments were conducted in which IAA (10^{-5} M and 10^{-4} M) was supplied in the solution at the cut end of the coleoptile. This was done by keeping the cut end in the IAA solution for durations of 2 and 4 hours. The lengthy IAA treatments were thought to be necessary as the previous results had shown that the whole of the coleoptile was perceptive to the red-far red stimulus and at an estimated transport rate of 1 cm per hour it would take 4 hours for the IAA to move down the 4 cm length of the coleoptile. While the IAA treated coleoptiles had visibly grown more than the untreated decapitated

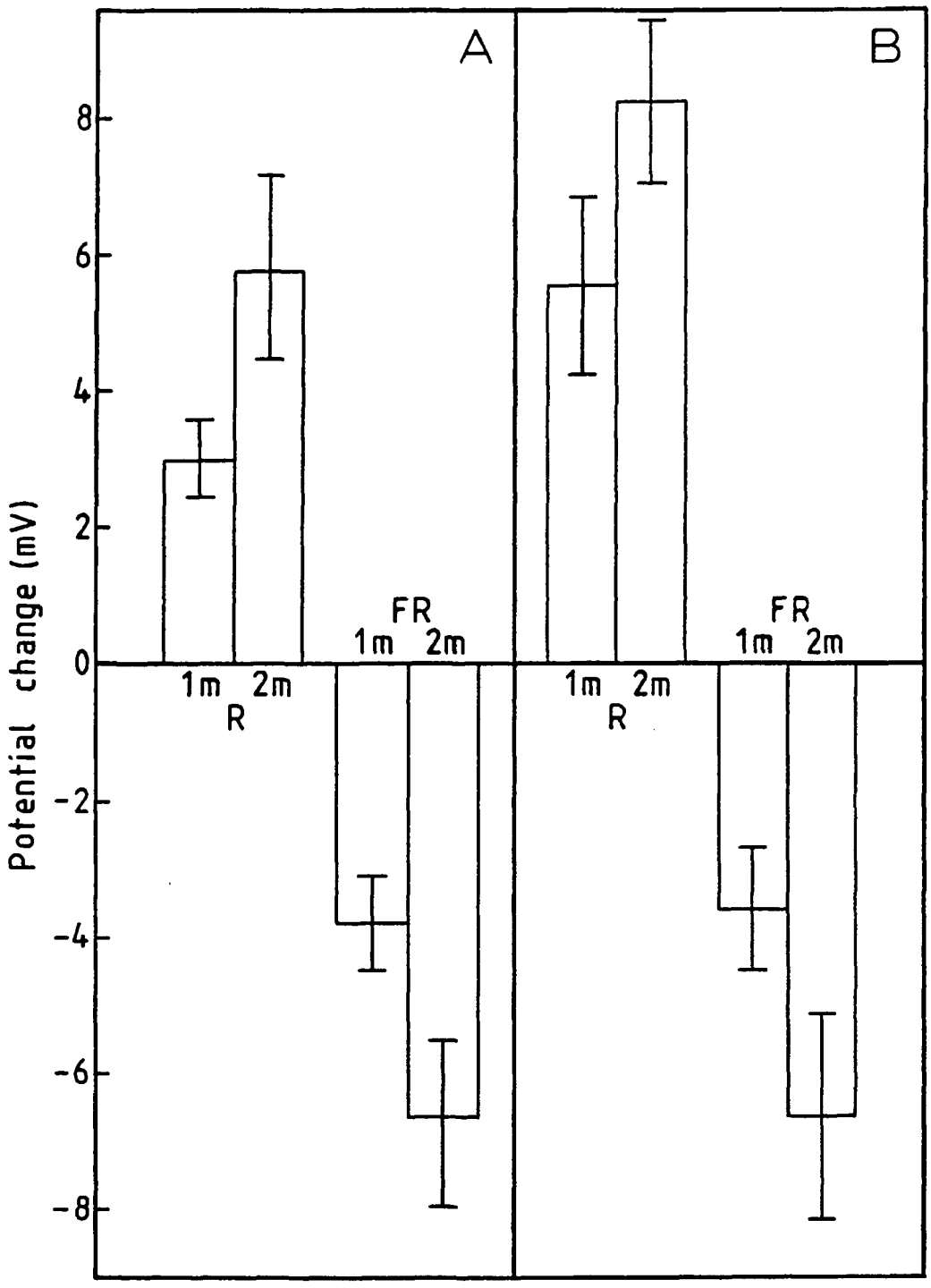


Fig. 3.5 Average potential changes following either 1 or 2 minutes alternate R and FR treatments, of (A) intact seedlings (B) seedlings with decapitated coleoptiles (used within 25 minutes of decapitation). The results are means ± SE (n = 5).

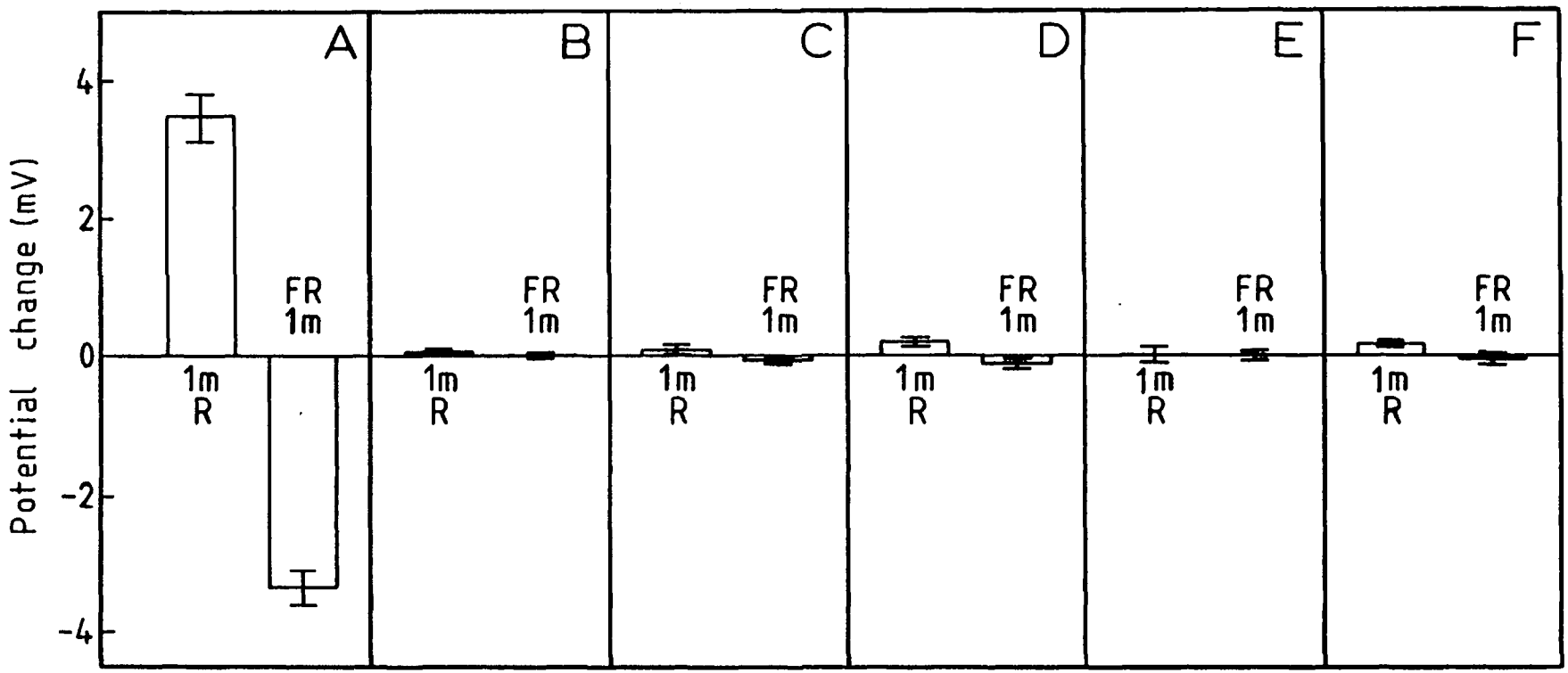


Fig. 3.6 Average potential changes, following alternate 1 minute R and 1 minute FR treatments, of seedlings with coleoptiles (A) intact, (B) one hour after decapitation, (C) decapitated and the cut ends kept in 10^{-5} M IAA for 2 hours, (D) decapitated and the cut ends kept in 10^{-5} M IAA for 4 hours, (E) decapitated and the cut ends kept in 10^{-4} M IAA for 2 hours and (F) decapitated and the cut ends kept in 10^{-4} M IAA for 4 hours. The results are means ± SE (n = 5-9).

coleoptiles, the results depicted in Figure 3.6 (C-F) show that IAA, at concentrations which caused the growth of the coleoptile, did not substitute for the tip in maintaining the phytochrome-mediated potential changes.

3.2 Spontaneous Electrical Activity in Plants Grown in Light

3.2.1 Preliminary Experiments

Marrow, pumpkin and cucumber plants were used initially in order to discover whether these plants showed any spontaneous electrical activity resembling action potentials. Marrow plants were found to be more active in this respect and therefore, they were used in most of the remaining experiments. The electrical activity was recorded between the reference electrode, placed in the plant pot and one or two recording (tungsten or Ag/AgCl) electrodes, placed on the aerial parts of the plant. A description of the various types of activity observed follows.

3.2.2 Repetitive Spiking

The most interesting electrical activity seen in marrow and pumpkin plants were the spikes which occurred repetitively. Figures 3.7, 3.8, 3.9, 3.10 and 3.11 show some of the trains of signals observed in marrow plants. Most of the trains shown were recorded from very young leaf petioles (leaf area about 5 cm^2). The signals in these trains had the following characteristics.

3.2.2.1 Shape of Fluctuations

In general the signals showed a rapid rise in potential and then a slow fall to the base-line. In particular, the initial 10% rise was much more sudden than the final 10% return. The rise time (measured between the attainment of 10 and 90% amplitude) was typically between 150 and 350 ms. That this was not limited by the response time of the recorder was checked. The trains shown in Figures 3.9D and 3.10C are the only exceptions to this general trend

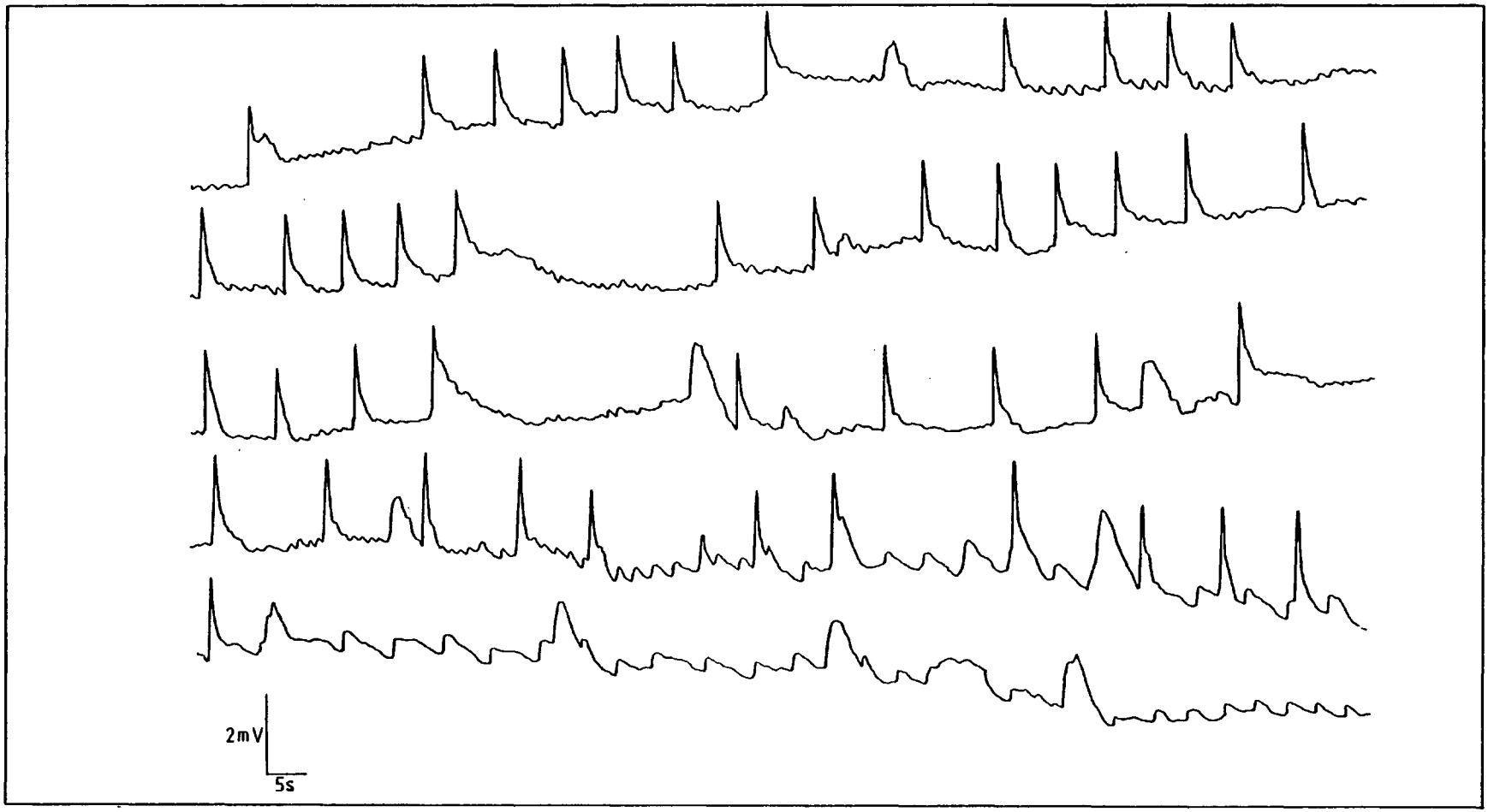


Fig. 3.7 An unbroken sequence of repetitive signals recorded by Ag/AgCl electrode on the petiole of a medium-sized leaf using a D.C. coupled amplifier.

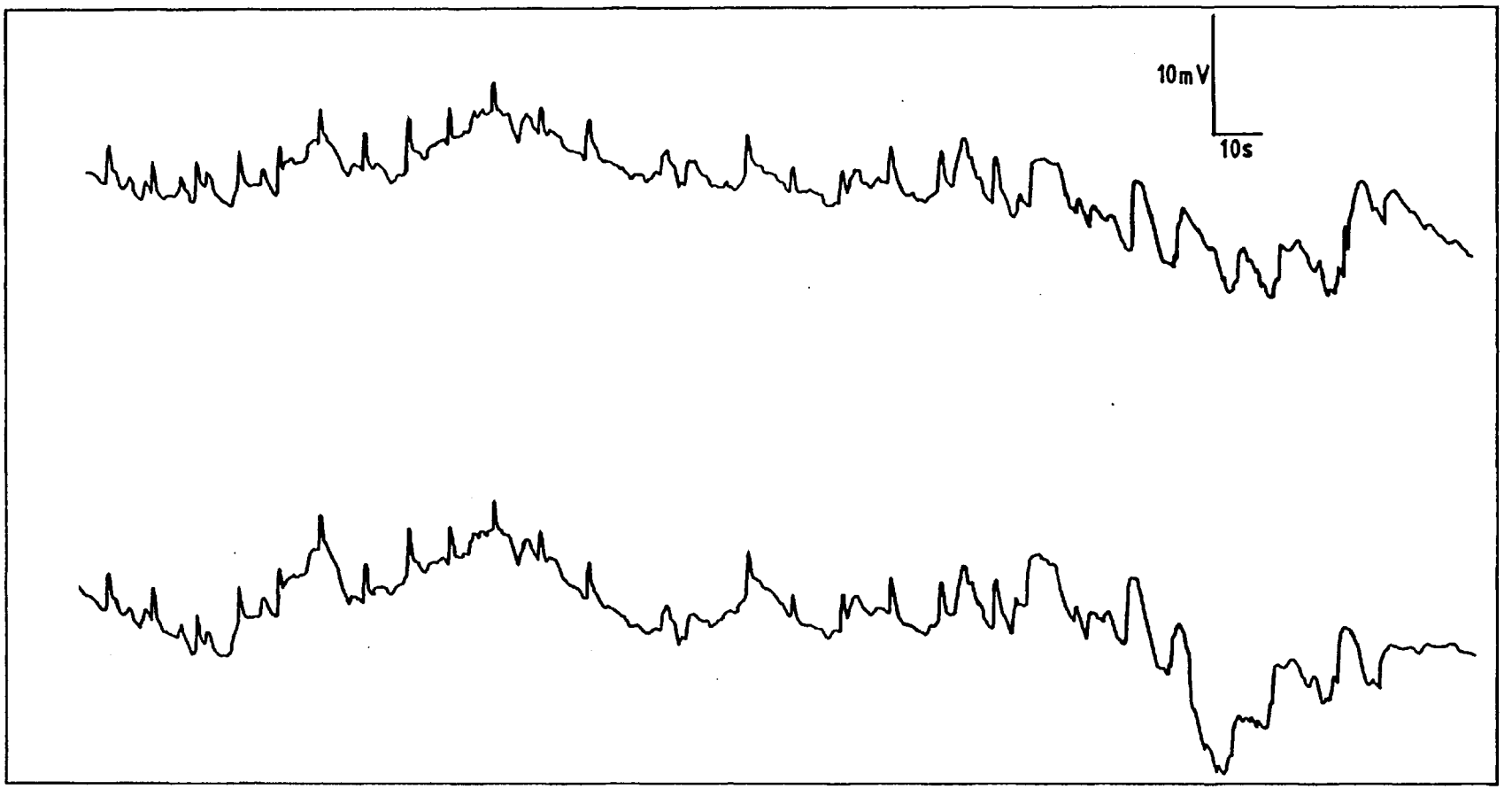


Fig. 3.8 A two-channel recording, made with A.C. coupled amplifiers, of a train of repetitive signals showing their synchronous occurrence on each channel. The recording tungsten electrodes were placed 2 cm apart in the petiole of a young leaf.

as the signals do not show a clear difference between the rise and the fall time. At times, near the end of a train the signals start showing a shoulder (Figure 3.7). The duration (indexed as time between attainment of and return to 10% of the maximum amplitude) of signals, with a rapid rise and slow fall, typically ranged between 0.5 to 2 seconds.

3.2.2.2 Amplitude and Polarity

Although, normally the amplitude of signals within a train did not show much variation, in some cases (Figure 3.9C and 3.10B) there were large variations. The amplitude of the largest fluctuations observed in a train was 4 mV (Figure 3.9A) and the smallest being 0.25 mV (Figure 3.9B). In almost all cases the signals were positive going. The signals recorded synchronously at two points which were close together always had a similar shape and amplitude (Figure 3.8 and 3.9).

3.2.2.3 Frequency

The frequency of the signals varied from train to train and sometimes within a train. It could be quite regular as seen in Figure 3.9B or very irregular as seen in Figure 3.7. The frequency of spiking in various trains ranged from 5 to 16 per minute. The start or the end of a train could be abrupt, although at times the frequency went down before the end of a train. Acceleration or deceleration of spiking could occur at any point in the train.

3.2.2.4 Uniformity of Fluctuations in a Train

In the majority of cases, most signals within a train were of similar shape and size but occasionally, some trains contained

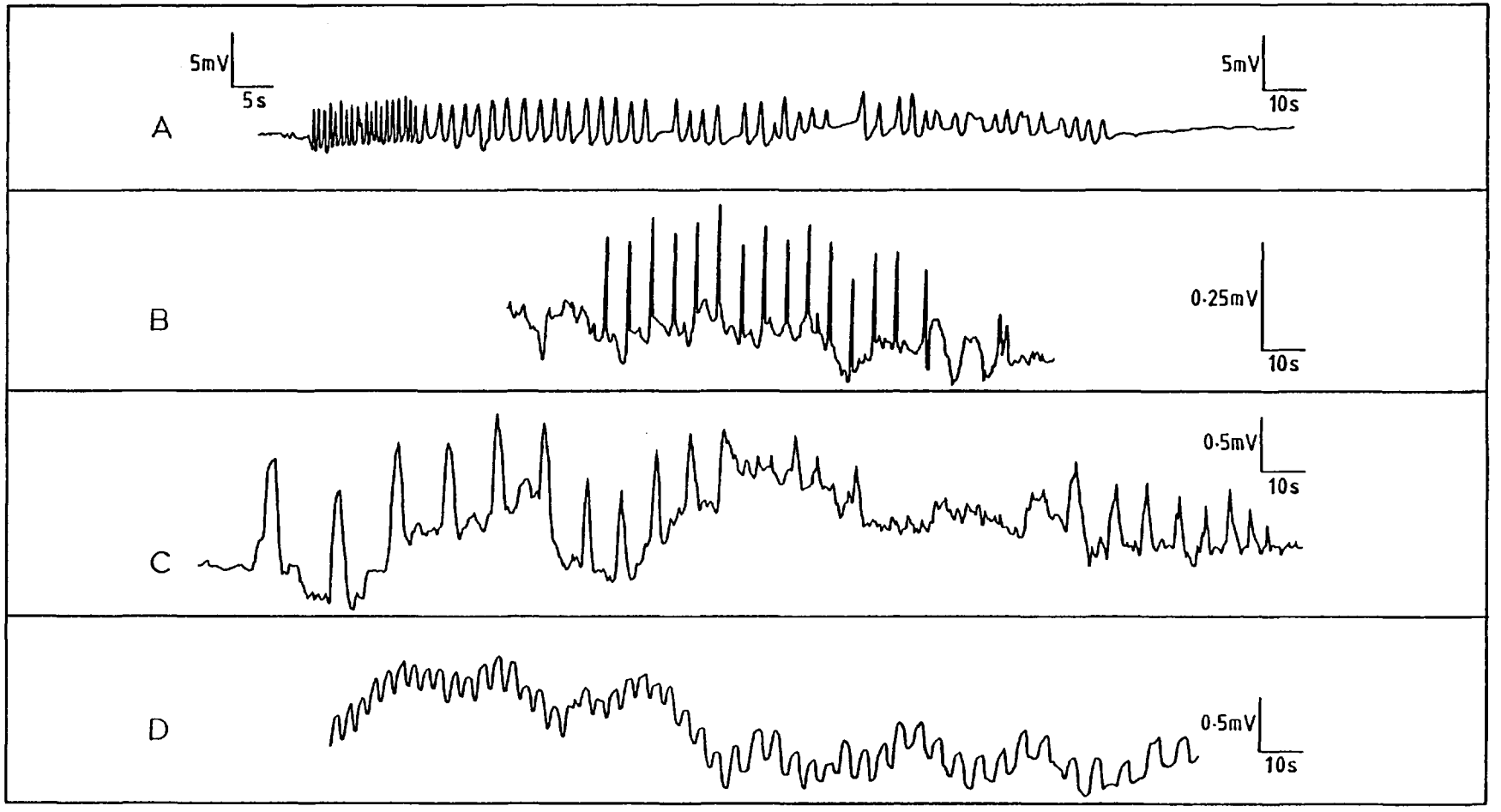


Fig. 3.9 Four different trains of signals which were picked up simultaneously by two tungsten electrodes placed between 1 and 4 cm apart. A to C were from young petioles and D from a young stem. A.C. coupled amplifiers were used in each case.

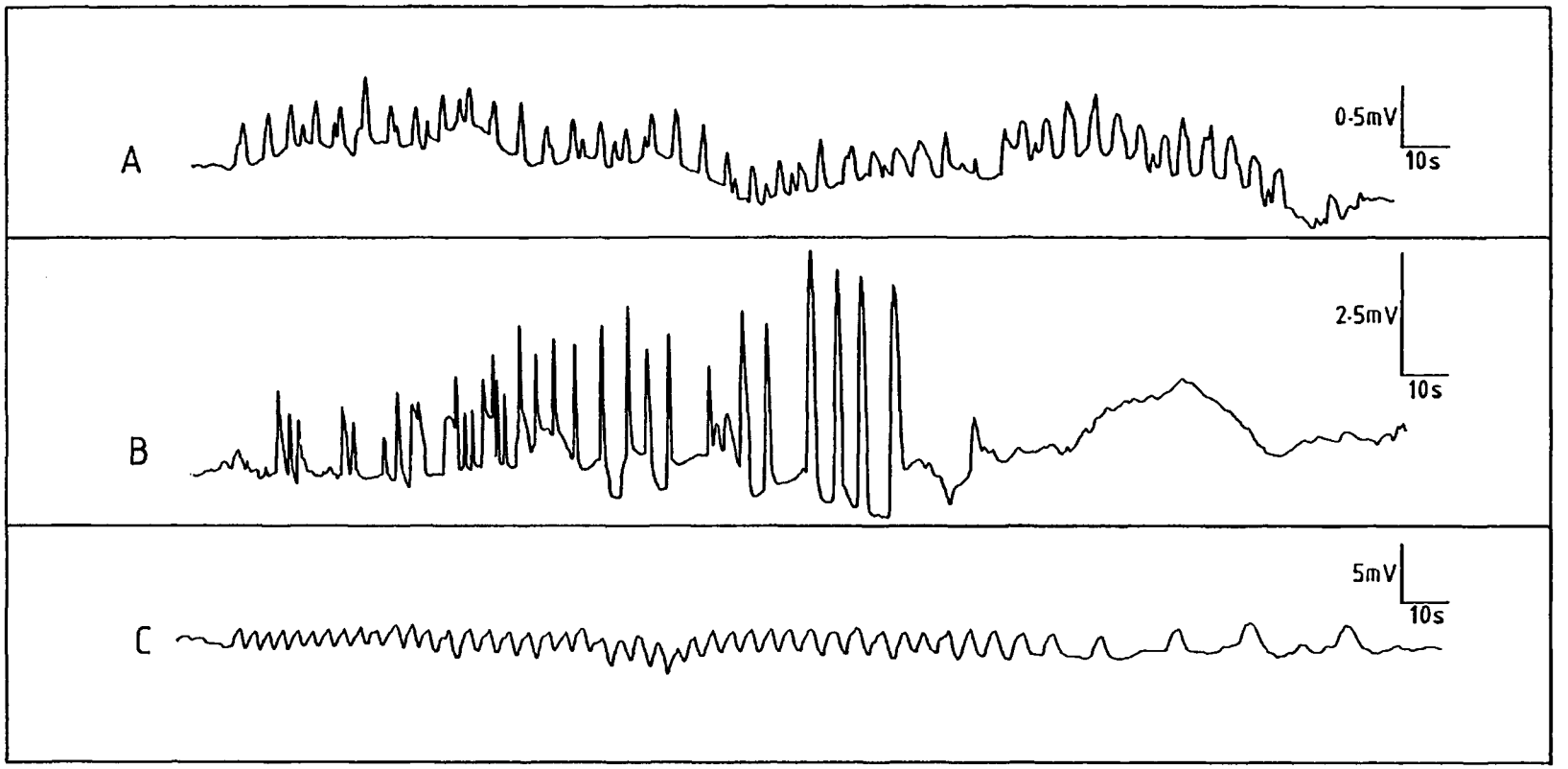


Fig. 3.10 Three different trains of signals picked up by A.C. coupled amplifiers and a tungsten electrode placed on the petiole of a young leaf, but not detected by another electrode placed on a more mature part of the plant.

spikes which showed no uniformity (Figure 3.9C and 3.10B).

3.2.2.5 Train Duration

The shortest train observed lasted 76 seconds (Figure 3.9B) and the longest one lasted for more than 10 minutes (Figure 3.7), but it is quite possible that trains of shorter or longer durations occur from time to time.

3.2.2.6 Site of Occurrence

The repetitive signals were detected in the petioles and younger growing parts of the stem (near the last leaf). Most of the recordings shown were obtained from the petioles of young growing leaves. Only in one case (Figure 3.7) were repetitive spikes observed in a mature leaf petiole. This suggests the association of these signals with growth. When these signals occurred they could be picked up simultaneously by two electrodes placed as far as 4 cm apart (Figure 3.8 and 3.9). When the distance was greater, these signals were usually picked up only by the electrode nearest the growing region (Figure 3.10). Thus, it seems that the signals can be detected close to their site of origin, but, as they were picked up synchronously at two separate points within this region, they seem not to show true propagation.

In order to check whether these signals were of plant origin and not a result of electrical interference, in the middle of an experiment when the plant was showing a train of signals, a 10 M resistor was placed between the input terminals of the amplifier in place of the plant and the recording continued for some time before reconnecting the plant. Figure 3.11 shows that the activity disappeared when the resistor was substituted for the plant and reappeared

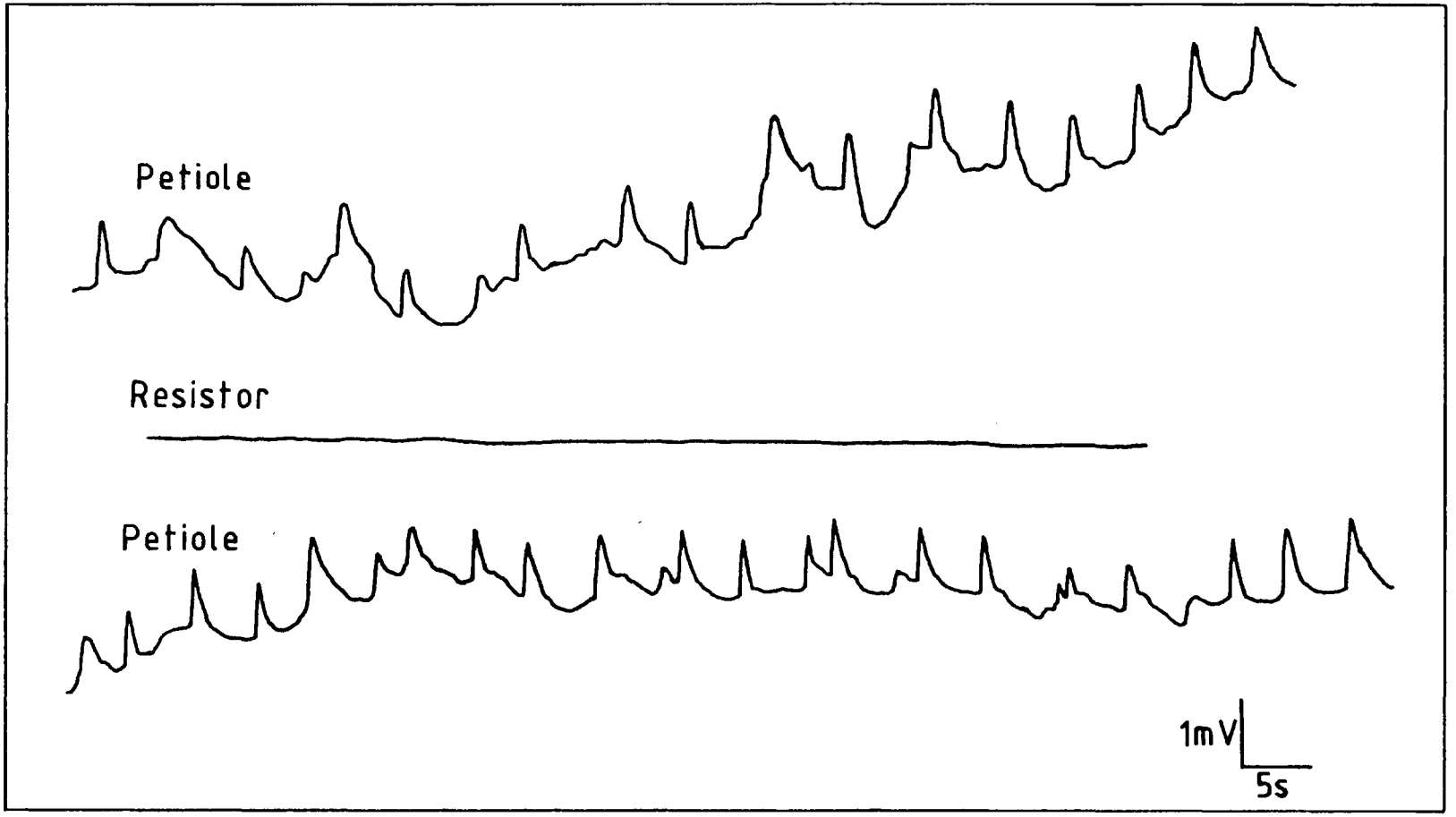


Fig. 3.11 Evidence that the signals are of plant origin. The trace shows a recording made with a D.C. coupled amplifier of a train of signals which was interrupted when a 10M resistor was connected in place of the plant for one minute.

when the electrodes were reconnected to the plant, suggesting that the activity was of plant origin. The fact that the repetitive signals were detected by only one of the two recording electrodes placed far apart on the plant (Figure 3.10) also indicates that the activity was not due to electrical interference, since such noise would in general be seen in each of the two neighbouring, identical recording circuits.

3.2.3 "Irregular" Fluctuations

Fluctuations, relatively larger than the ones described in the previous section, also occurred spontaneously. There was a great variation in the shape and size of signals within a recording (Figure 3.12). Their amplitude ranged from 1 to 10 mV. Signals of one or both polarities could be seen in a single recording. Occasionally, recordings showed fluctuations of up to 40 mV of either polarity. The fluctuations did not always have rapid rise and slow fall, sometimes their rise time was nearly comparable to their return. The duration of these signals ranged from 0.5 to 5 seconds. Within a recording, periods of high activity and quiet periods could be seen. Two electrodes placed as near as 1 cm on the plant did not show any common fluctuations. They always showed different activity. These fluctuations could be detected in younger or mature parts of the plant with tungsten as well as Ag/AgCl electrodes.

3.2.4 Complex Low Level Activity

Most of the time, as illustrated in Figure 3.13, recordings made at higher sensitivity showed a complex but low level of electrical activity. It appeared to result from the superposition of numerous individual fluctuations of various sizes. The amplitude of

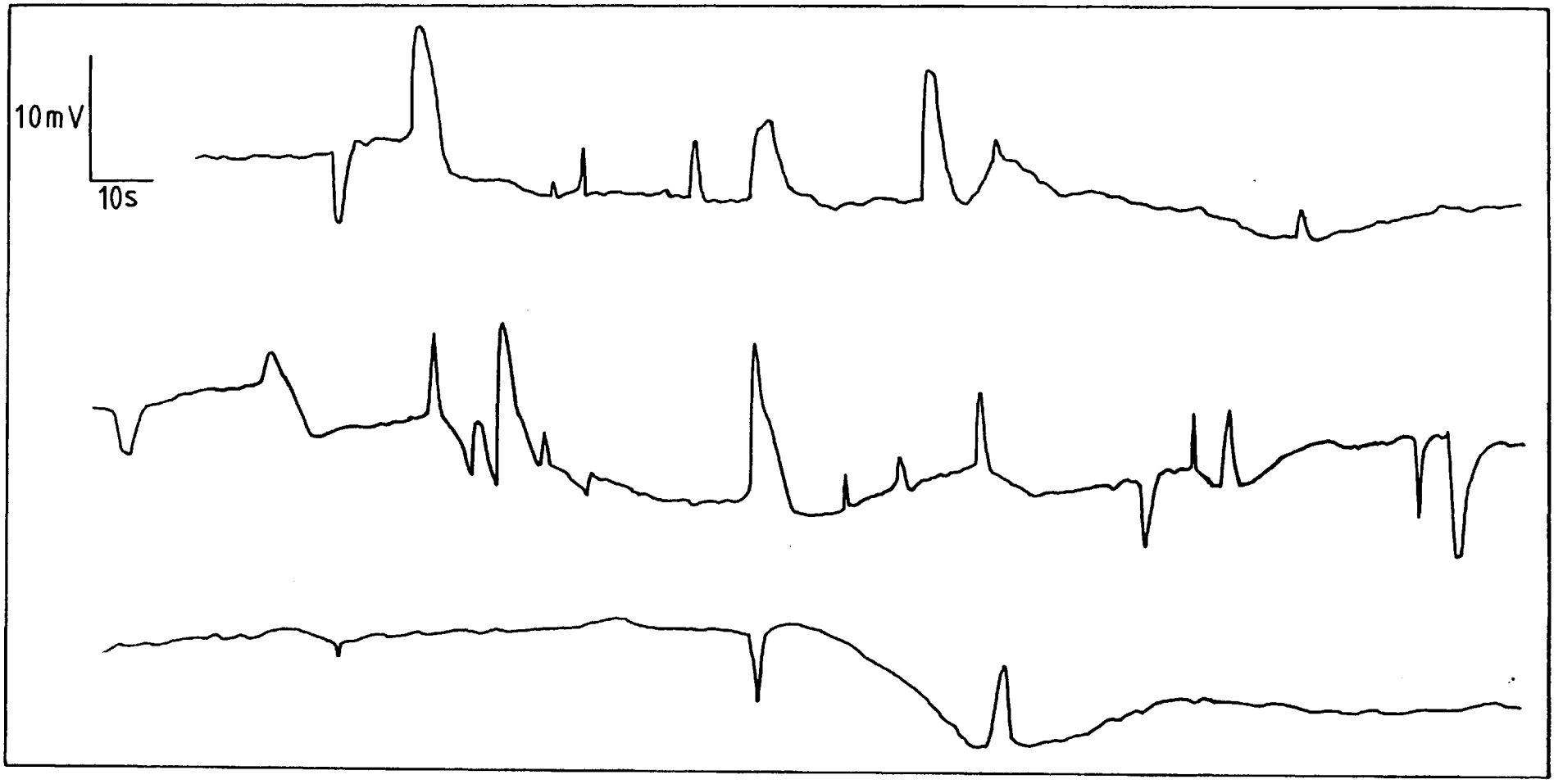


Fig. 3.12 An unbroken recording showing "irregular" fluctuations in a petiole. A tungsten electrode and D.C. coupled amplifier was used.

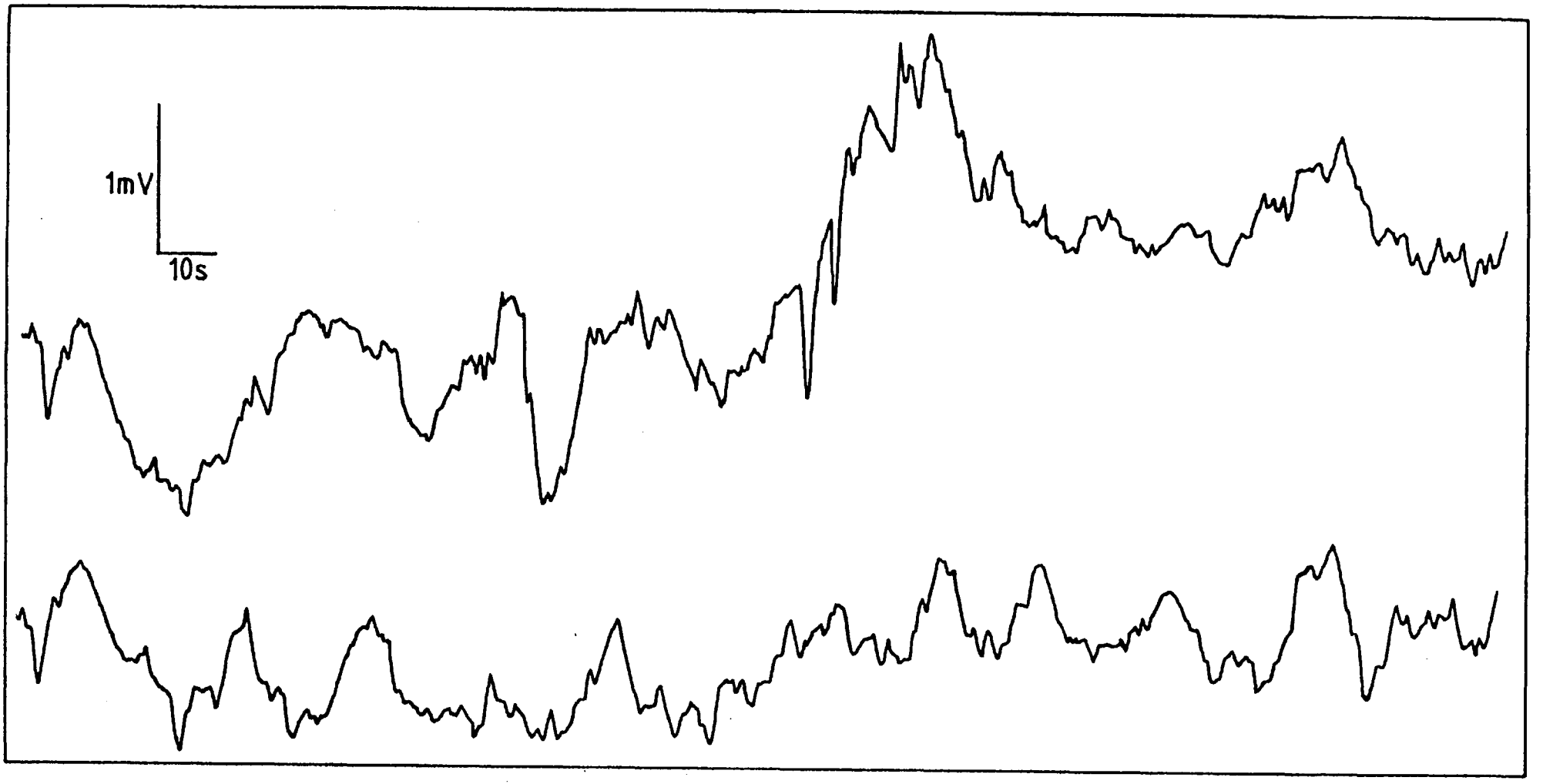


Fig. 3.13 Complex activity recorded simultaneously with A.C. coupled amplifiers and two Ag/AgCl electrodes placed 2 cm apart on a petiole.

these groups of fluctuations could be as high as 10 mV. This type of activity might last from a few minutes to many hours. A recording might show periods of high activity with intervening periods of low activity. A part of the plant showing this kind of activity might suddenly start showing repetitive spiking or the "irregular" fluctuations and vice versa. At times some parts of the complex activity were picked up simultaneously on two electrodes placed about 1-2 cm apart. Figure 3.13 shows recordings from two electrodes placed 2 cm apart which have partial similarity. Complex activity recorded at two points more than 2 cm apart did not show any common features. That the activity was not electrical noise was checked using a 10 M resistor as described before.

3.2.5 "Solitary" Signals Recorded at Two Different Points Simultaneously

These signals were very rare and picked up simultaneously by two recording electrodes placed more than 10 cm apart on the aerial parts of the plant. Figure 3.14 illustrates parts of some recordings which showed such signals. These signals were large (8-35 mV) and positive-going. As seen from the figure the background complex activity on two traces was entirely different at the time of occurrence of the signal. The rarity of these signals precluded their being tested for plant origin by using a resistor as a dummy plant. However, if they are of plant origin, their simultaneous occurrence at widely distant points suggests their transmission by electrotonic conduction rather than by true propagation. In many ways, these signals resembled those detected in the stem resulting from electrical disturbances in the root following treatment with KCl.

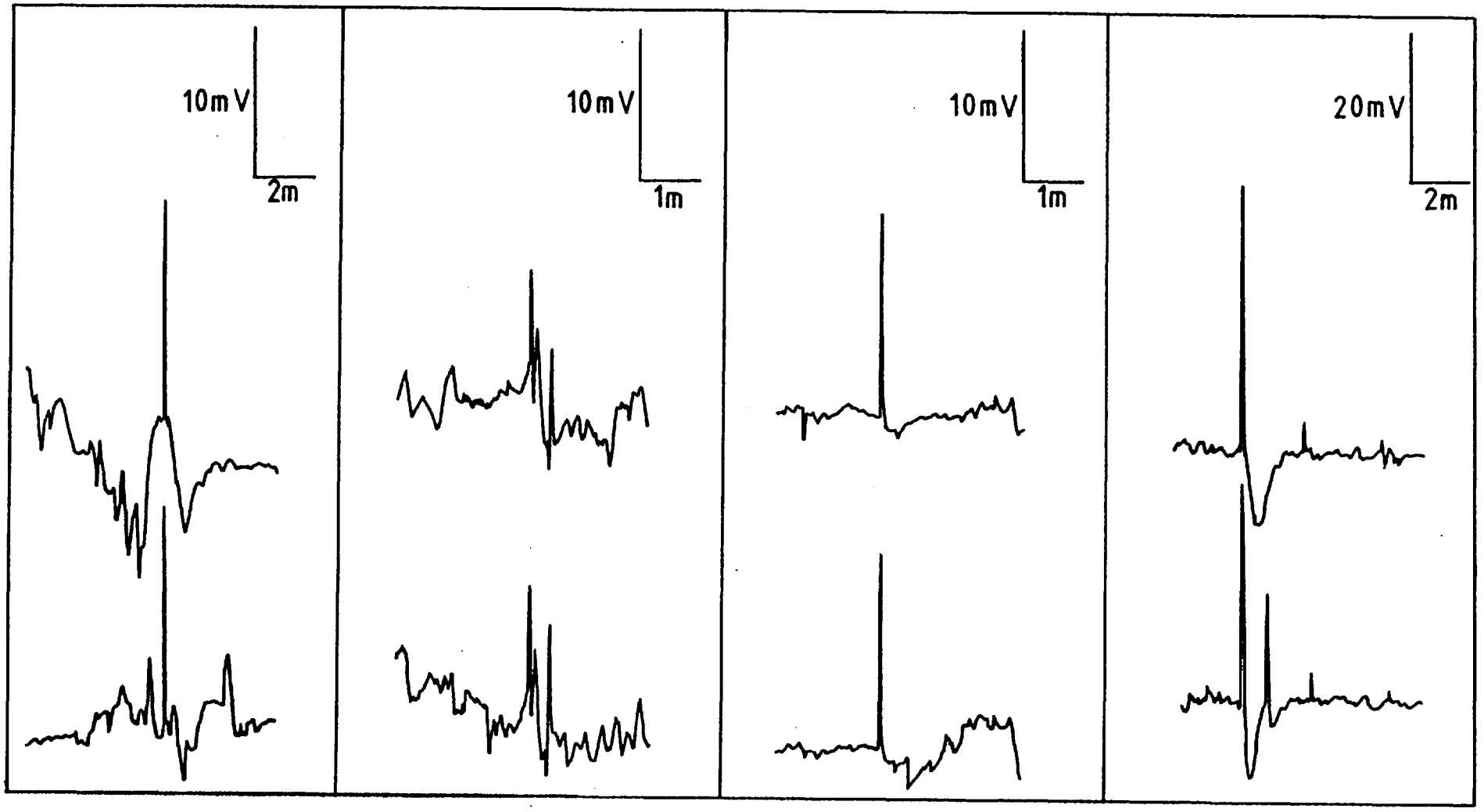


Fig. 3.14 Examples of large positive-going "solitary" signals recorded simultaneously with A.C. coupled amplifiers and tungsten electrodes placed over 10 cm apart on the aerial parts of the plant.

3.3 Electrical Activity Induced as a Result of a Stimulus

3.3.1 Effect of Different Light Conditions

Experiments were conducted on marrow plants in order to examine the effects of different light conditions on their electrical activity, in particular the repetitive spiking. This was because Spanner (1979) had predicted that this type of activity might be a consequence of electroosmotic flow in the phloem. Since phloem translocation is dependent on light, a study of electrical activity under different light conditions might provide some clue to its association with translocation. An extensive series of experiments was carried out in which a plant or a part of the plant was subjected to light or dark treatments. Figure 3.15 shows some typical results. The electrical activity of plants kept in the dark for up to 4 days did not differ very much from the activity seen before the dark treatment. Complex electrical activity as described in the previous section or sometimes activity with "irregular" fluctuations were seen. In further experiments, a single leaf of the plant was kept in the dark, by covering it with aluminium foil, in the greenhouse for varying periods before the plant was used for electrical recording. There was no obvious difference between the electrical activities recorded from the petioles of the leaves before and after they were kept in dark for up to 4 days (Figure 3.16). Exposure of a single leaf or the whole plant to light had no evident effect on the electrical activity of the petiole in the plants which had been kept in the dark for a day (Figure 3.17).

All these experiments with dark and light treatments were conducted using leaves of various sizes, and similar results were obtained. Thus it seems that light or dark conditions, which

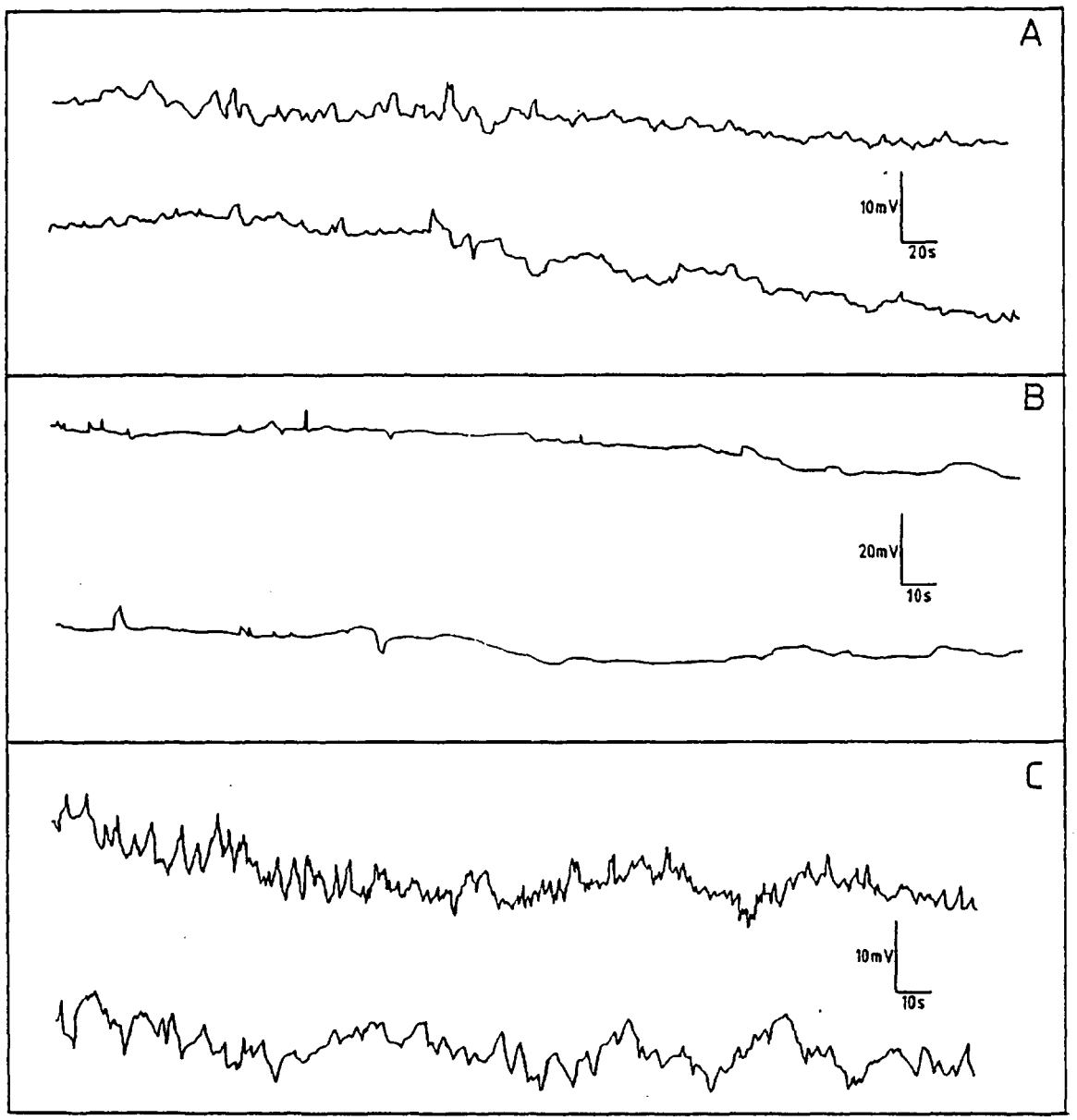


Fig. 3.15 Dark treatment of the whole plant. The upper trace in each case is a recording of electrical activity before the plant was kept in the dark. The lower traces are recordings made after the plant was kept in dark for (A) 1 day, (B) 2 days and (C) 4 days. The recordings were taken from the leaf petioles with Ag/AgCl electrodes and D.C. coupled amplifiers.

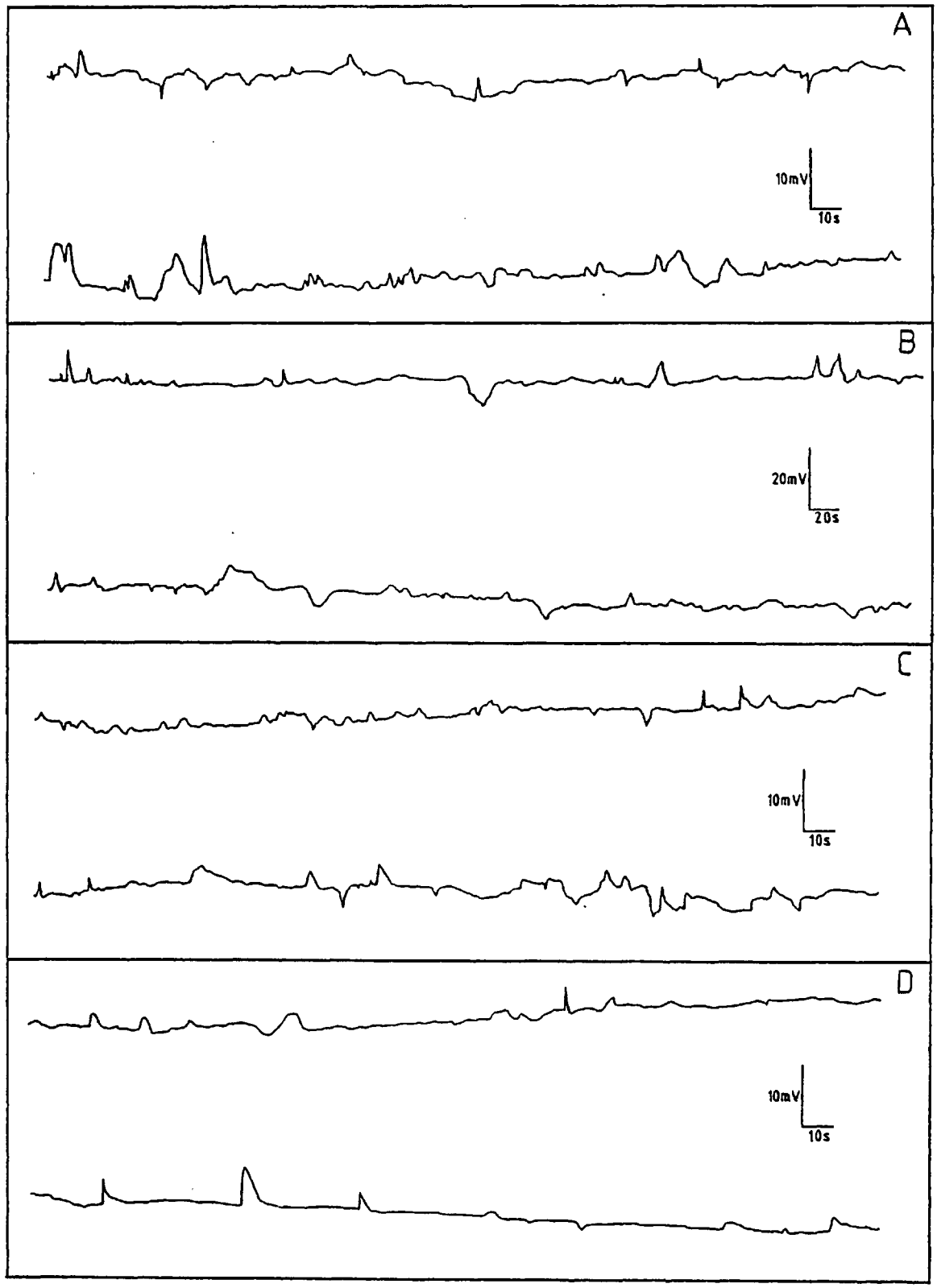


Fig. 3.16 Dark treatment of a single leaf. The upper trace in each case is a recording of electrical activity before the leaf was kept in the dark. The lower traces are recordings made after the leaf was kept in the dark for (A), (B) 2 days; (C), (D) 4 days. The recordings were taken from the leaf petioles with Ag/AgCl electrodes and D.C. coupled amplifiers.

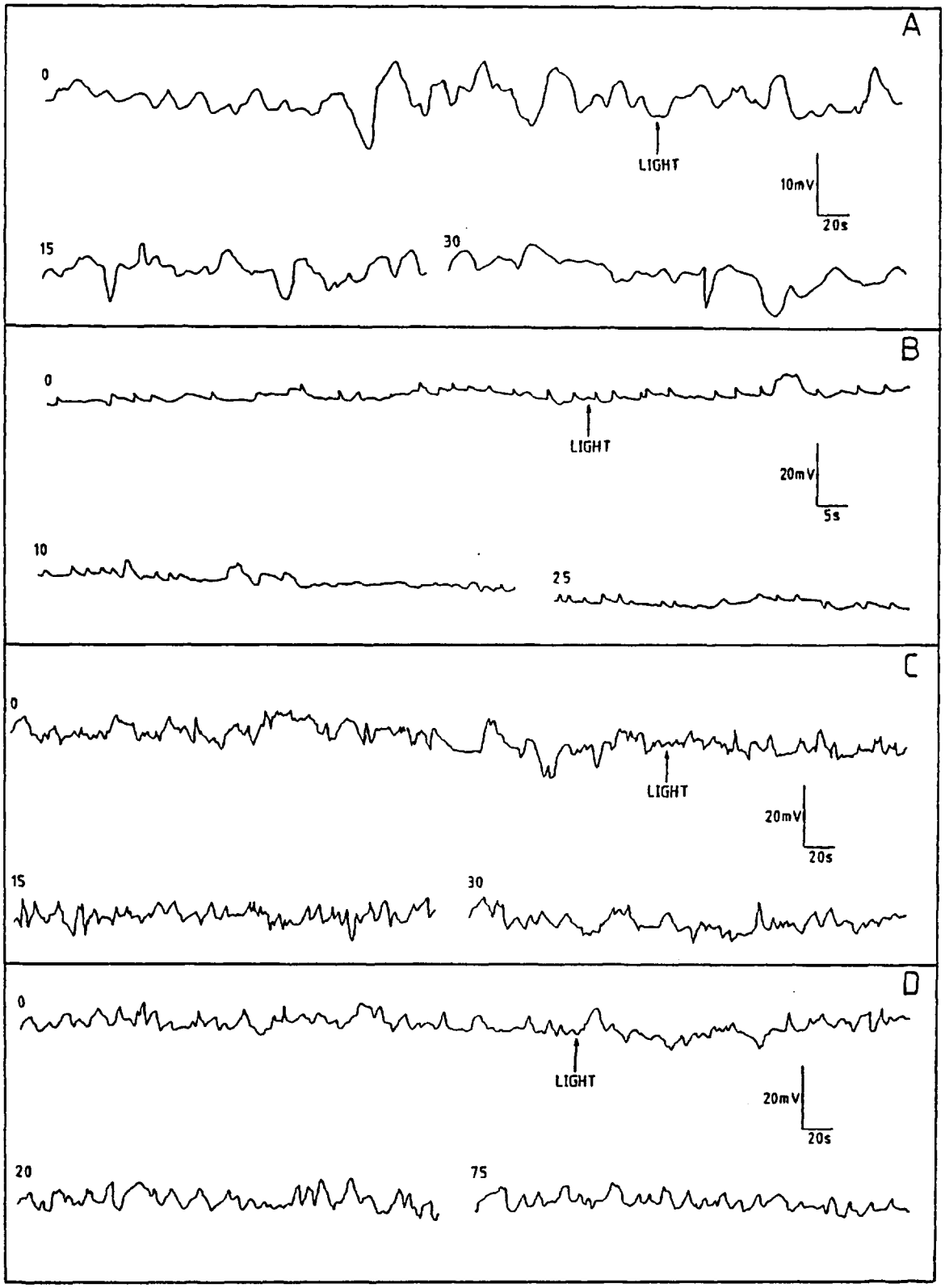


Fig. 3.17 Light (30W.m^{-2}) treatment of the plant. In (A), (B) the whole plant was exposed to the light; in (C), (D) a single leaf was exposed to the light. 0 represents, in each case, the start of a recording and the other numbers represent the time (minutes) after the start. The recordings were taken from the leaf petioles with tungsten electrodes and A.C. coupled amplifiers.

normally affect photosynthesis and phloem translocation, had no effect on the electrical activity.

Some further investigations, under different light conditions, with fine tungsten electrodes placed in or near the vascular bundles, showed no large (50 mV) electrical activity which should be associated with the phloem (Spanner, 1979) if electroosmosis is the driving force for phloem translocation.

3.3.2 Chemical Stimulus at the Leaf of Marrow Plant

As pointed out in section 3.2 the trains of repetitive spiking were mainly detected in the petioles of very young leaves or in the younger parts of the stem. Therefore, it was thought that IAA, which would be at a higher concentration in the young growing parts of the plant, might in some way be responsible for the fact that the trains were generated more frequently here. Also, it was thought that IAA, which is known to affect membrane permeability and cell membrane potentials, would have some effect on the electrical activity of the part of the plant to which it was applied. Figure 3.18 showed some of the recordings taken from the petiole, before and after the leaf was treated with IAA. Only in one case did the electrical activity increase after the IAA (5.10^{-6} M) treatment. However, further experiments with the same and higher concentrations of IAA did not show any effect. Therefore, another series of experiments was conducted in which IAA was applied to the petiole near the electrode in the form of a droplet. The results illustrated in Figure 3.19 show that local IAA treatment did not alter the electrical activity. Thus it seems that IAA does not have short-term effects on the electrical activity. However, its long-term effects cannot be ruled out on the basis of these experiments. Synthetic

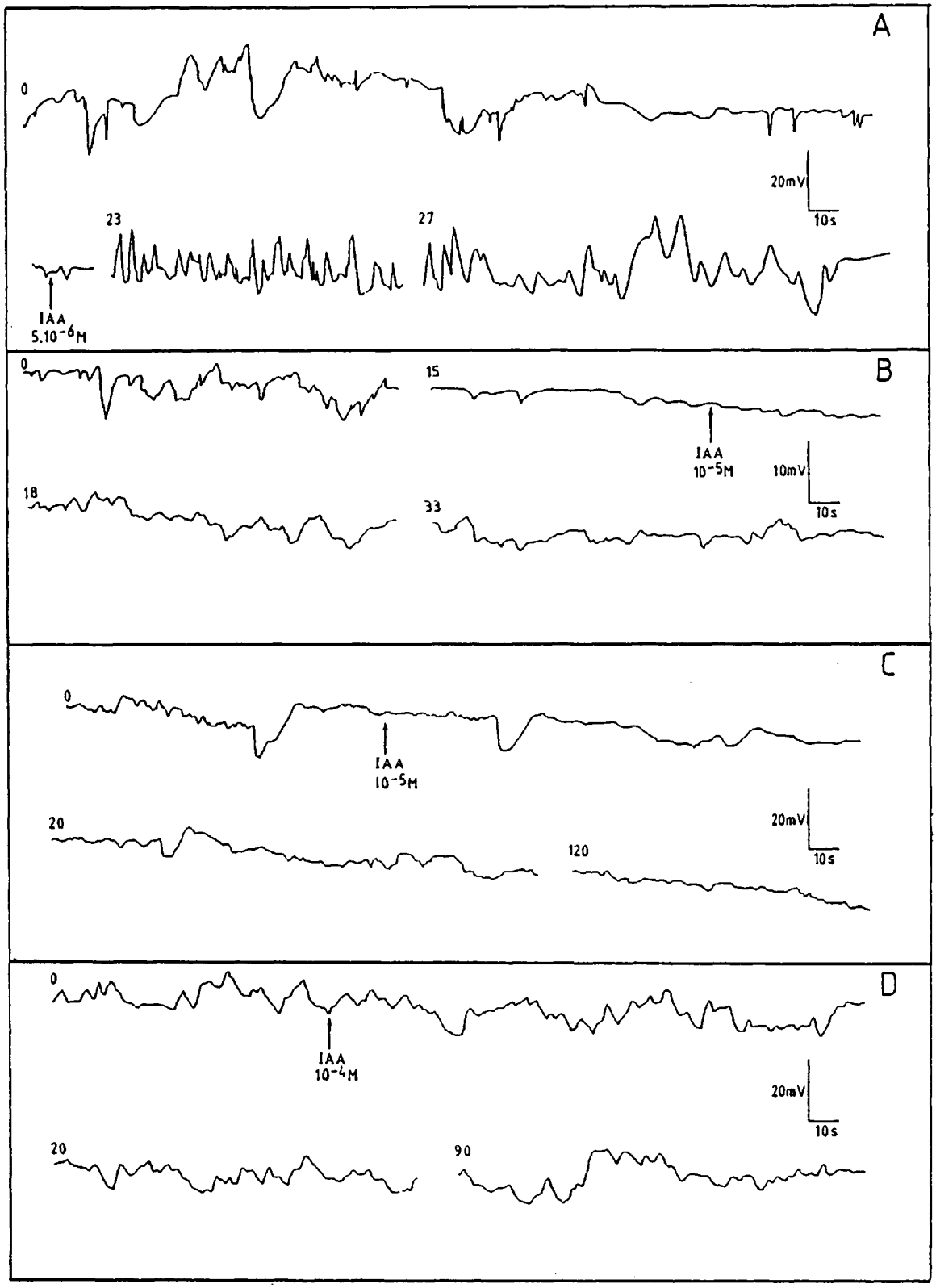


Fig. 3.18 IAA treatment of a leaf. IAA was applied to the leaf in the form of a spray. 0 represents, in each case, the start of a recording and the other numbers represent the time (minutes) after the start. A tungsten electrode, placed in the petiole at a distance of about 2 mm from the lamina, was used to record the activity. The amplifiers were D.C. coupled.

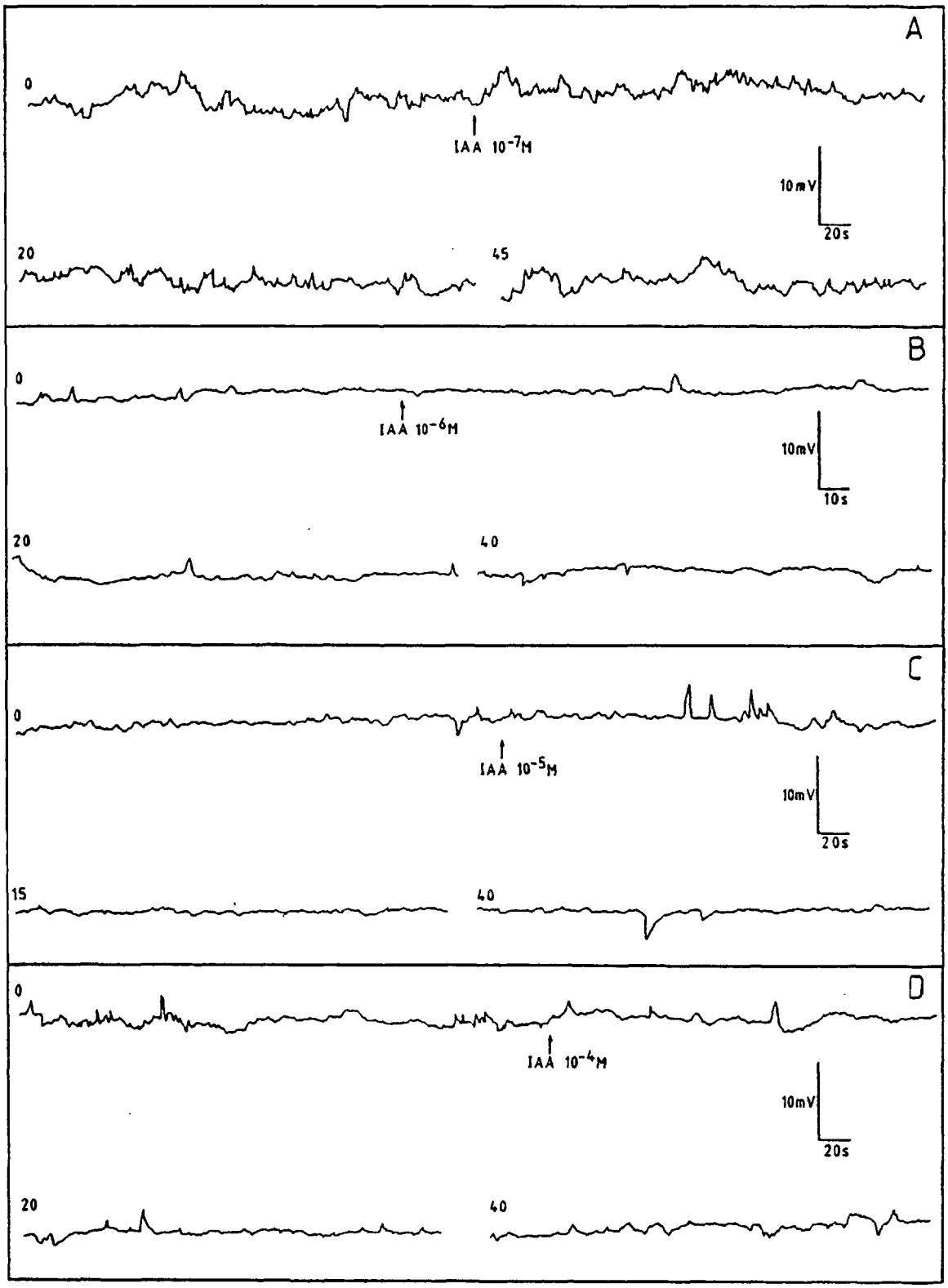


Fig. 3.19 Local IAA treatment. A drop of IAA was applied to the petiole near the recording tungsten electrode. 0 represents, in each case, the start of a recording and the other numbers represent the time (minutes) after the start. The amplifiers were A.C. coupled.

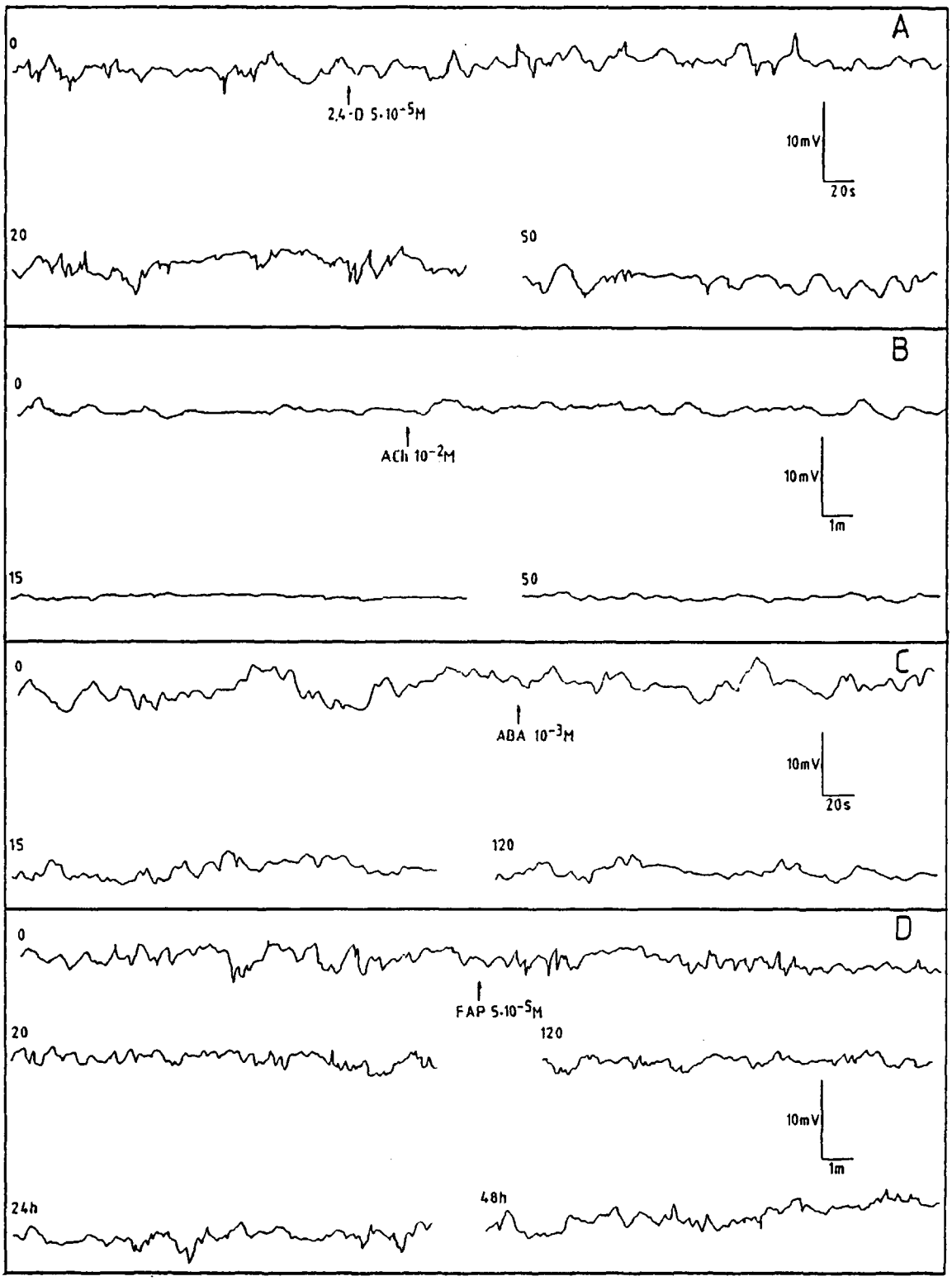


Fig. 3.20 (A) 2, 4-D, 5.10^{-5} M; (B) ACh, 10^{-2} M; (C) ABA, 10^{-3} M and (D) Kinetin (FAP), 5.10^{-5} treatment of the leaf. These were applied in the form of a spray to the leaf. 0 represents, in each case, the start of a recording and the other numbers represent the time (minutes, except the bottom trace in which the numbers are hours) after the start. The recordings were taken from the leaf petioles with tungsten electrodes and A.C. coupled amplifiers.

auxin 2,4-D, applied to the leaf also had little effect (Figure 3.20A).

Acetylcholine, which is known to affect cell membrane potentials and permeability (Jaffe, 1970), when applied to the leaf, did not have any effect on the electrical activity of the petiole (Figure 3.20B).

Abscisic acid, which induces closure of stomata in the leaves by controlling ion fluxes (Jones and Mansfield, 1970; Kriedemann et al., 1972) was also tried, but, no change in the electrical activity of the petiole was observed as a result of its application to the leaf (Figure 3.20C).

Kinetin (FAP) which induces transport of numerous substances including photosynthates to the leaf to which it is applied (Shindy and Weaver, 1970; Leopold and Kawase, 1964) should also affect phloem activity indirectly and therefore, it was tested to examine its effect on the electrical activity of the treated leaf petiole. After 2 days of its application the treated leaf was visibly more green and it looked as if it had drawn most of the nutrients and metabolites from the neighbouring leaves. However, the electrical activity recorded over this period did not show any obvious changes (Figure 3.20D), thus indicating once again that the electrical activity seen in the petiole is not associated with phloem transport.

3.3.3 Thermal Stimulus

In order to understand the nature of the electrical activity generated as a result of thermal stimulus and especially to differentiate this activity from other kinds of activity (e.g. KCl induced signals), some experiments were performed using marrow and pumpkin plants. Both plants were found to be equally good in producing heat



Fig. 3.21 A tracing of electrical activity in a petiole, induced as a result of a thermal stimulus (indicated by the arrow) at the lamina. A tungsten recording electrode, placed in the petiole, was connected to D.C. and A.C. coupled amplifying circuits.

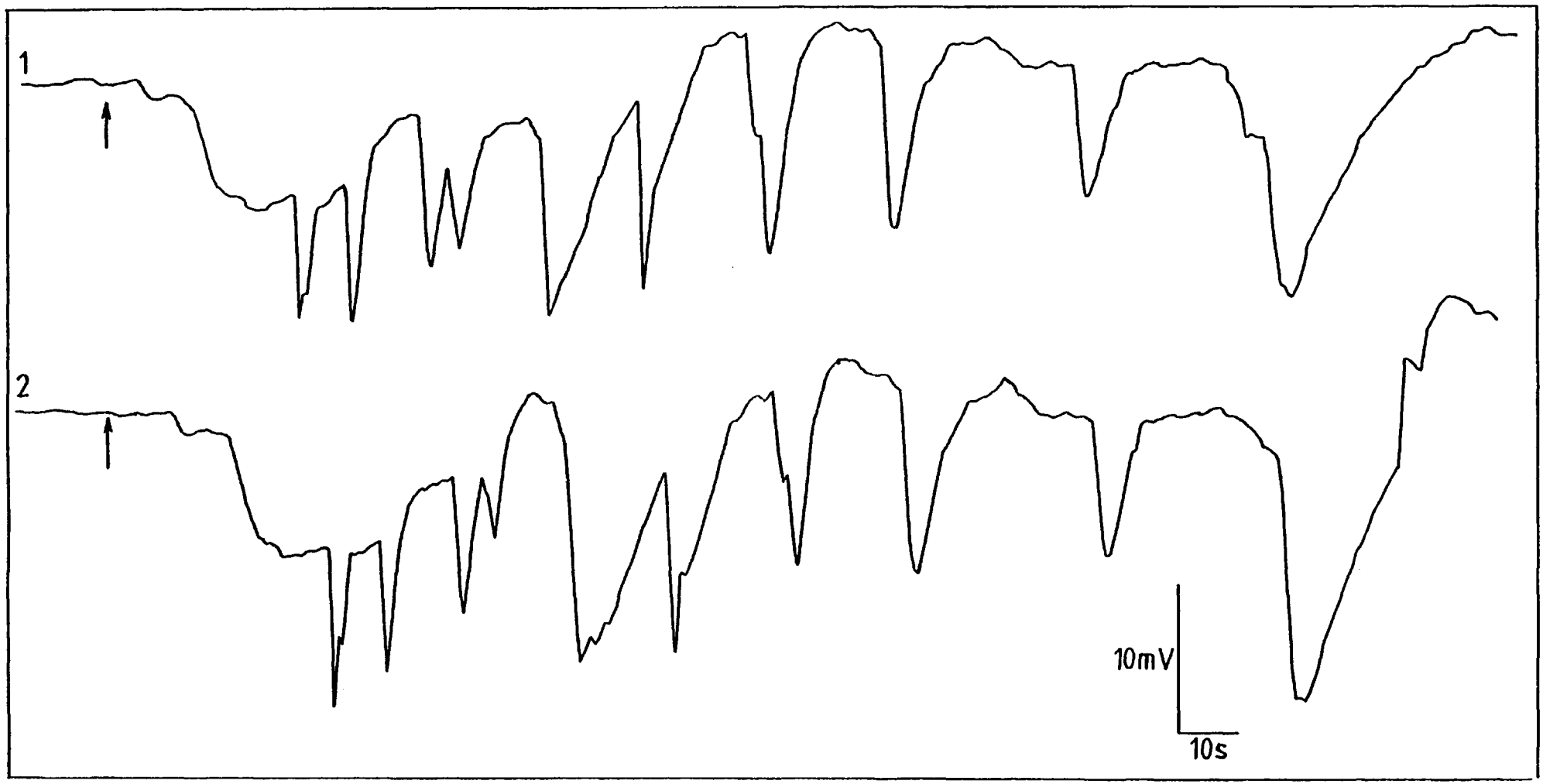


Fig. 3.22 An A.C. coupled recording showing basipetal propagation of signals in the petiole, induced as a result of a thermal stimulus (indicated by the arrow) at the lamina. Tungsten electrode 1 was placed on petiole near the lamina and tungsten electrode 2 was placed 6 cm further down. The rate of basipetal propagation as measured from the time delay between corresponding parts of each trace was about 10 $mm.s^{-1}$.

stimulated signals, although the recordings described in this section were all taken from marrow plants. The electrical activity was detected by two recording electrodes (tungsten or Ag/AgCl) placed on either the petiole or stem. The reference electrode was in the pot. Figure 3.21 shows the electrical fluctuations detected in the petiole after a part of the leaf was wilted by a burning match. The presence of A.C. coupling (a filter with a 20 seconds time constant) in the amplifying circuit did not significantly affect the shape of the fluctuations (particularly, the initial rising phase in both the cases were very similar). Therefore, further recordings were carried out with the A.C. coupling in the amplifying circuit because it eliminates base-line drift. The figure also shows that several signals were produced as a result of the stimulus. The signals were of negative polarity and their size varied from 10 to 35 mV. Figure 3.22 illustrates the propagating nature of these signals. The rate of basipetal propagation of the signals in a petiole ranged from 6-10 mm. s^{-1}. The time between the application of the stimulus to the leaf and the appearance of the signals in the petiole varied from 10 to 30 seconds. Large (30 mV or more) heat induced signals could pass through the node into the stem and spread acropetally and basipetally. These could be detected in other leaves situated above or below the stimulated leaf. At times, the heat stimulus at the leaf resulted into one large signal which might be associated with a series of small putative action potentials (Figure 3.23).

Some experiments were conducted to examine the electrical activity induced in the stem as a result of heat damage to the roots. A special reason for these experiments was to compare the heat stimulated signals with the signals produced in the roots due to other treatments (e.g. KCl). The two recording electrodes were placed at

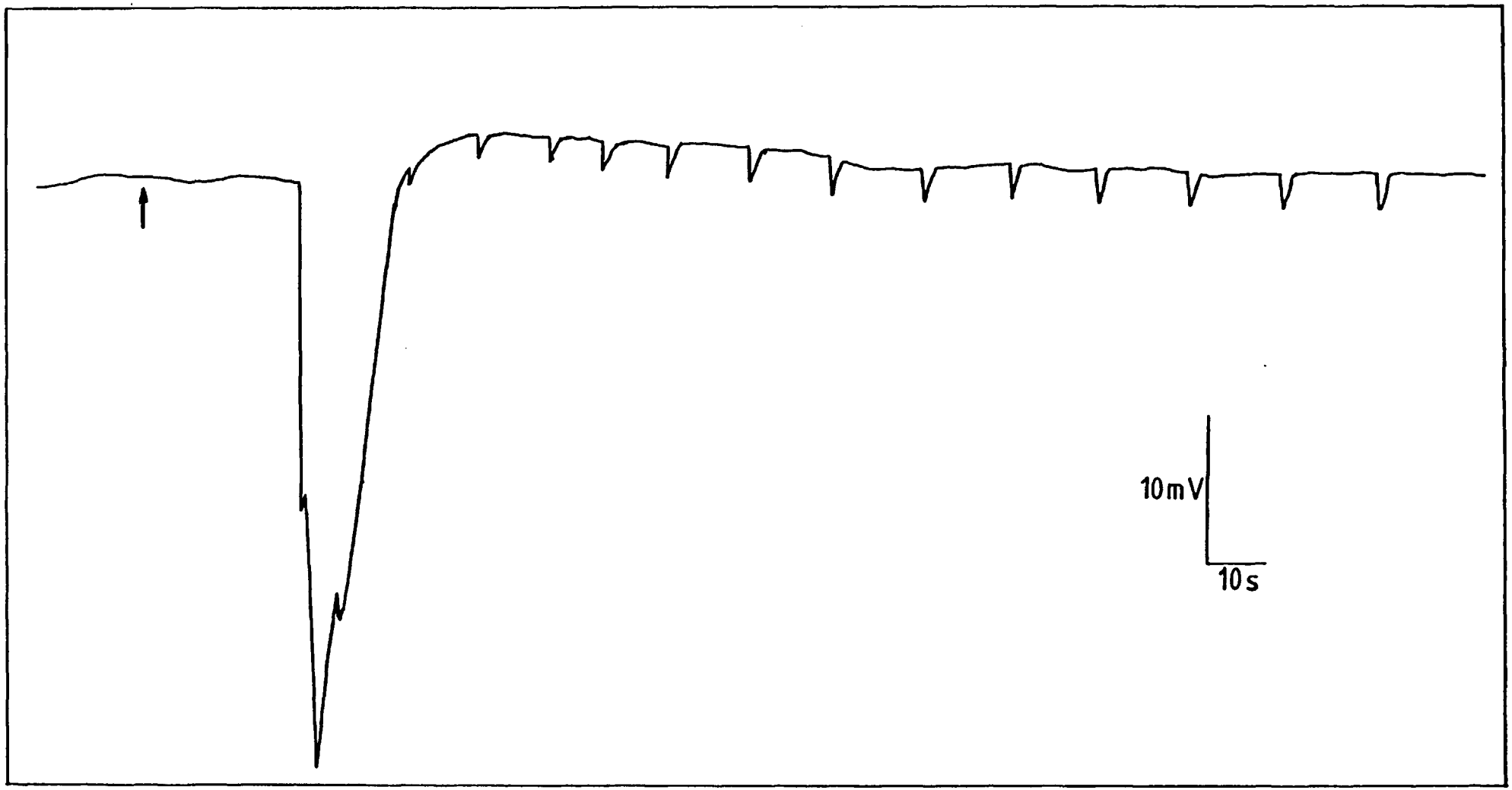


Fig. 3.23 A D.C. coupled recording showing electrical activity, picked up by a tungsten electrode placed in the leaf petiole, induced by a thermal stimulus (indicated by the arrow) at the lamina. It shows a large signal associated with small putative action potentials.

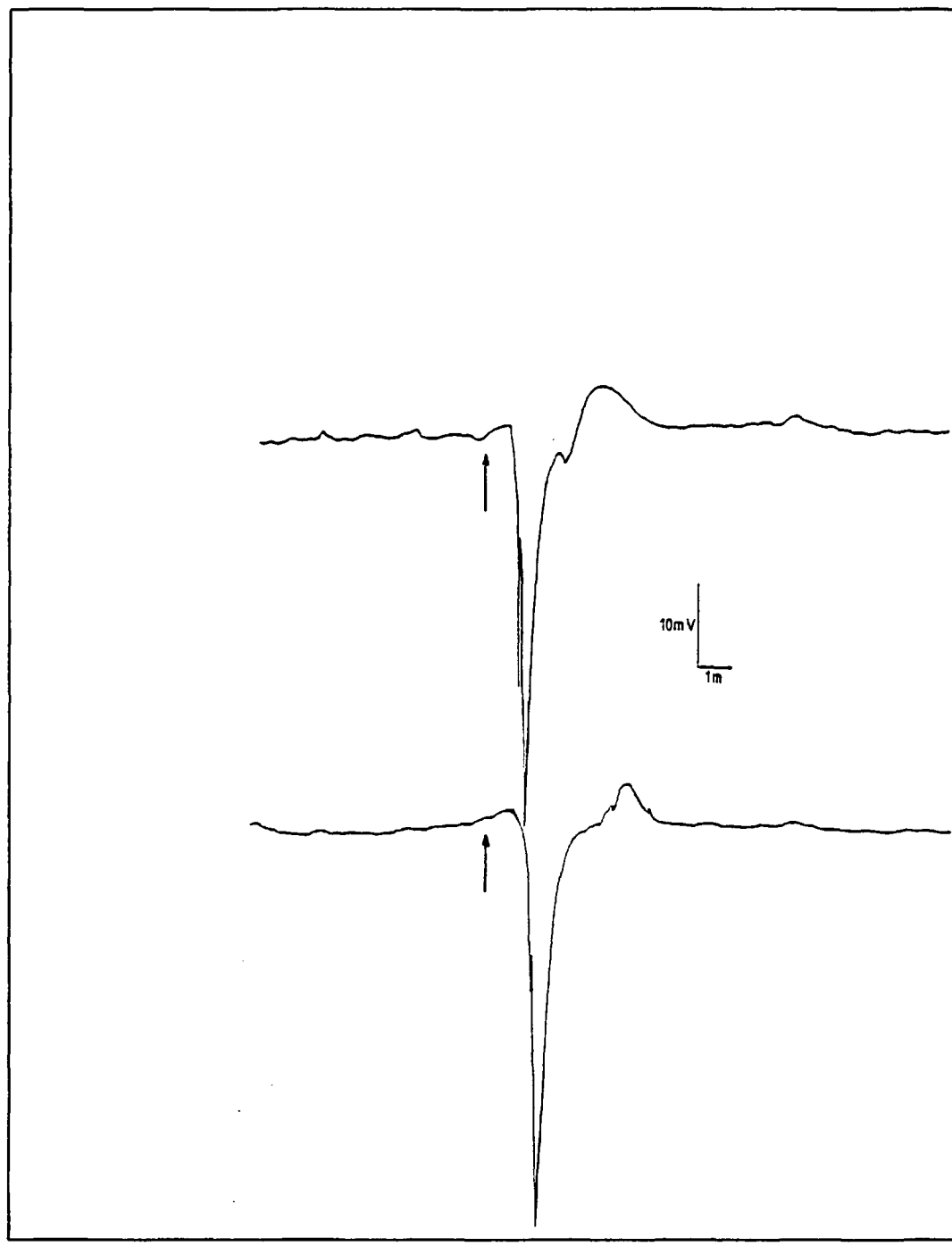


Fig. 3.24 Electrical activity, recorded in the stem by two tungsten electrodes placed 6 cm apart, induced by a thermal stimulus (indicated by the arrow) at the roots. The amplifiers were A.C. coupled. The rate of acropetal propagation of signals was about 3 mm.s^{-1}.

two points on the stem. The reference electrode was in the nutrient medium surrounding the roots. The stimulus was administered by replacing this medium with a similar one at 90°C. One or more signals could be seen as a result of this stimulus (Figure 3.24). The signals as detected in the stem were of negative polarity and propagated acropetally at a rate of about 3-5 mm. s^{-1}.

3.3.4 Stimulus at the Roots

An extensive series of experiments was carried out with marrow plants in order to discover if the roots could be induced to produce any action potentials, to understand the nature of these and to find out if they were produced in response to any natural changes in the environment which a plant could encounter during its life time.

3.3.4.1 KCl

Preliminary experiments with various treatments showed that KCl was the best agent in inducing action potentials in the roots. Marrow, pumpkin and wheat plants were used initially and all of them could be induced by KCl to produce such signals. However, the results quoted in this section are for marrow plants only. Early experiments were conducted with the reference electrode placed in the pot and two recording (tungsten or Ag/AgCl) electrodes placed at two different points on the aerial parts of the plant. About 4 ml of 3.8 M KCl shots were administered in the pot. This KCl must have diluted down considerably as the soil in the pot contained more than 100 ml of water. The stimulus initiated a series of signals. Figure 3.25 shows a D.C. and A.C. coupled recording of the electrical activity picked up by a recording tungsten electrode placed in a leaf

petiole. The KCl stimulus resulted in an immediate positive change in the D.C. level of the petiole (where the amplifier was A.C. coupled this appeared as a single spike followed by a return to the base-line at a rate determined by the 20 seconds time constant). After varying intervals, ranging from 30 seconds to 5 minutes in different experiments, action-potential-like signals were observed in both types of recording. In Figures 3.25 to 3.29 the initial change in D.C. level has been omitted and the trace begins just before the first action potential. Since, the action potentials as seen on D.C. and A.C. coupled recordings were almost identical, most of the recordings were carried out with the amplifier being A.C. coupled to eliminate base-line drift. Figure 3.26 shows that the KCl-induced signals were picked up synchronously by two recording electrodes placed as far as 15 cm apart on the aerial parts of the plant which suggested that they did not show the slow propagation characteristics of plant action potentials but were instead probably transmitted by electrotonic conduction. The loss of amplitude of the signals during electrotonic conduction in the shoot was very slight and was barely noticeable even when the electrodes were placed as far as 15 cm apart (Figure 3.26). These signals, as picked up by the recording (tungsten or Ag/AgCl) electrodes placed on the aerial parts, were of positive polarity. The rise time typically ranged between 1 to 3 seconds. The duration of the signals ranged between 15 to 45 seconds and it could vary within a series. The peak amplitude typically ranged between 10 to 45 mV. Usually there was a gradual decline in the amplitude and the frequency of signals as the series progressed. The latent period between the stimulus and the first signal observed varied between 30 seconds and 5 minutes.

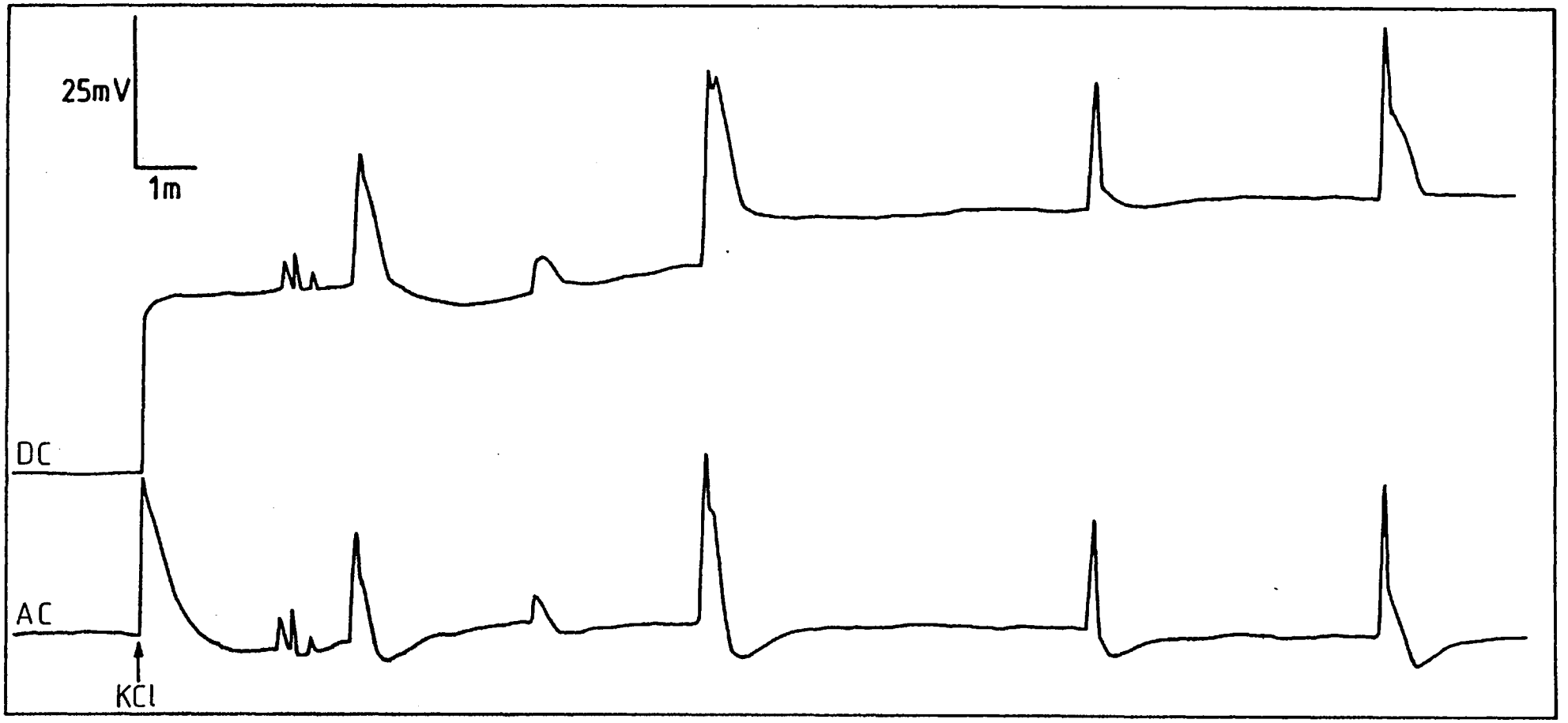


Fig. 3.25 A trace of electrical activity induced as a result of a KCl stimulus to the roots. A tungsten recording electrode placed on the petiole was connected to D.C. and A.C. coupled amplifying circuits.

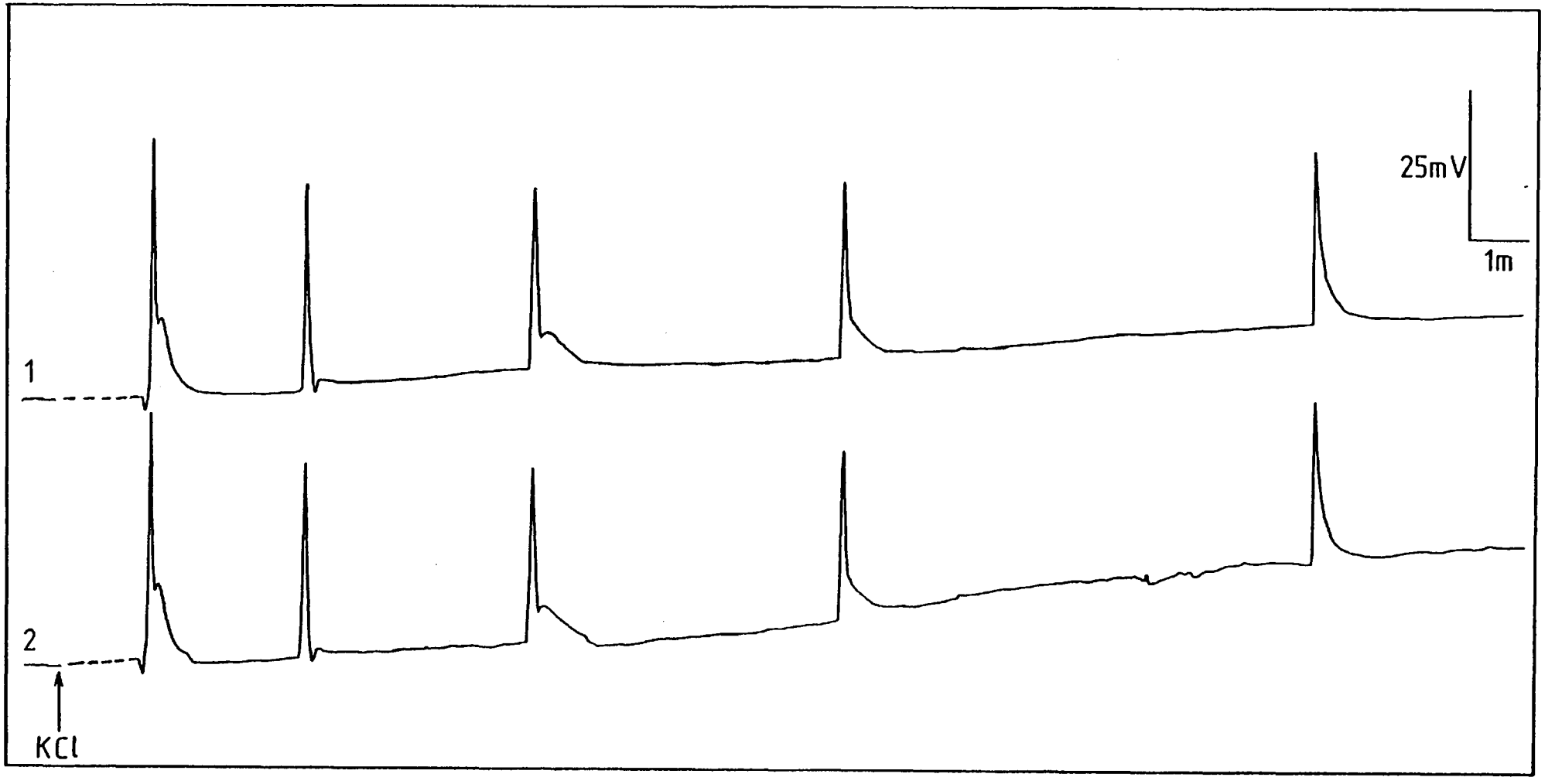


Fig. 3.26 A D.C. coupled recording showing synchronous detection of signals (induced by a KCl stimulus to the roots) at two different points by two tungsten electrodes placed 15 cm apart on the aerial parts of the plant. Electrode 1 was nearer to the roots.

A number of further experiments were performed in order to check if these KCl-induced signals showed any evidence of true propagation. The two recording electrodes placed as far as possible from each other at two points on the aerial parts of the plant always showed the appearance of signals synchronously, even at the highest chart speed (10 mm/s) of the recorder. In another experiment an electrode placed on a leaf blade, away from any large veins, showed synchronous appearance of signals similar in size and shape to those observed in the stem after a KCl stimulus (Figure 3.27A). Similar synchronous KCl-induced signals were also picked up in the tendrils (Figure 3.27B).

In order to investigate the possible role of the vascular bundles in transmitting the signals, some experiments were carried out in which the stem was cut off just below the first leaf, one tungsten electrode placed inside and another outside one of the exposed vascular bundles. Figure 3.28 shows that the polarity of signals inside and outside the vascular bundle was the same and that they were detected synchronously. However, the amplitude of the signals inside a vascular bundle was somewhat larger than outside (probably due to a greater ease of conduction within them). These results again indicate that the conduction of the KCl-induced signals to the remainder of the plant is electrotonic.

In one experiment a tungsten electrode inserted into the upper part of the main root was used to detect KCl-induced signals in them. Figure 3.29 shows that only the first signal was detected in the root, the rest were seen in the stem alone. It is interesting that the signal as picked up in the root was of negative polarity although it still occurred synchronously with the first signal

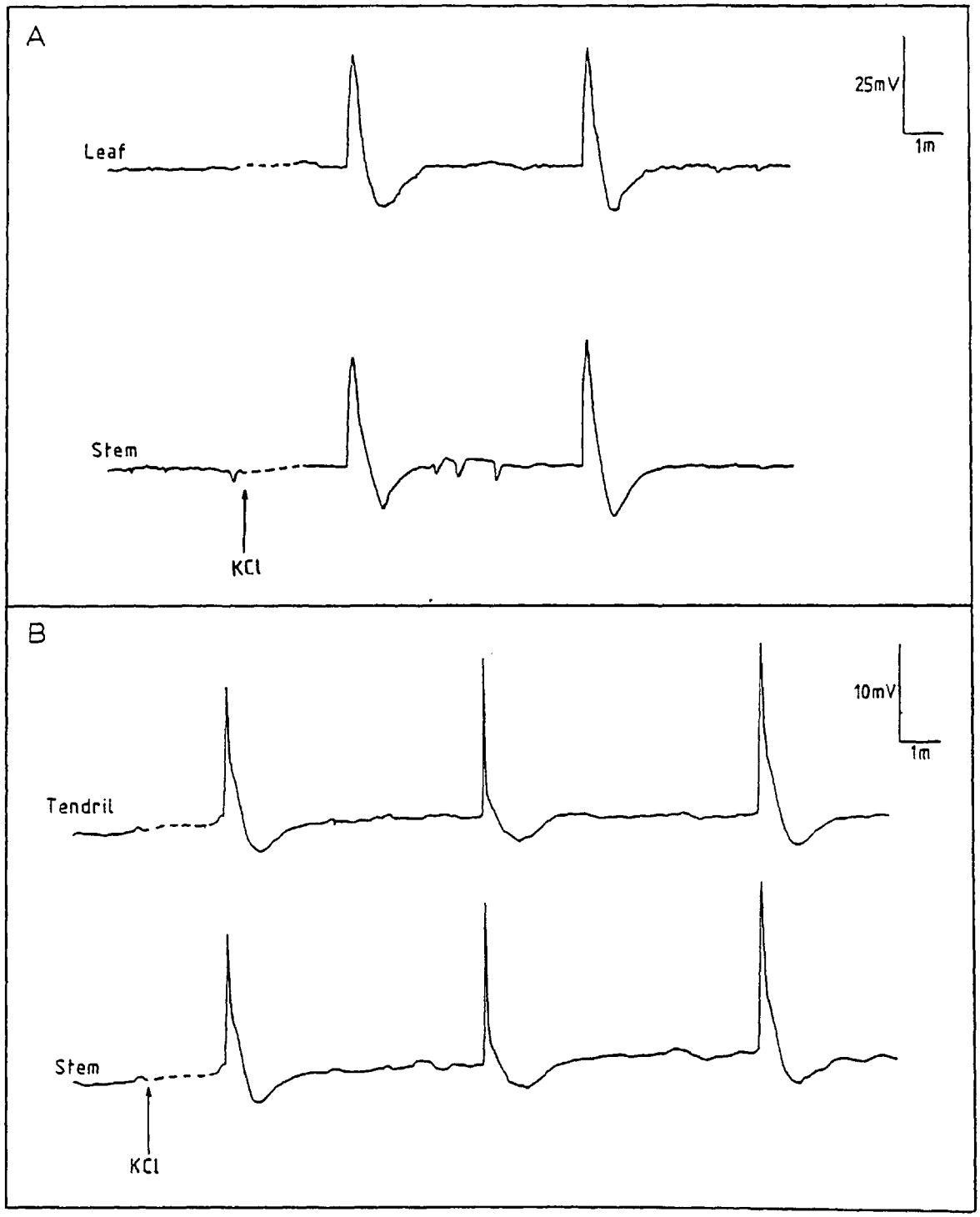


Fig. 3.27 Electrical activity induced by KCl applied to the roots. The recordings show synchronous detection of signals by two Ag/AgCl electrodes placed on (A) the leaf blade and the stem, (B) the tendril and the stem. The amplifiers were A.C. coupled.

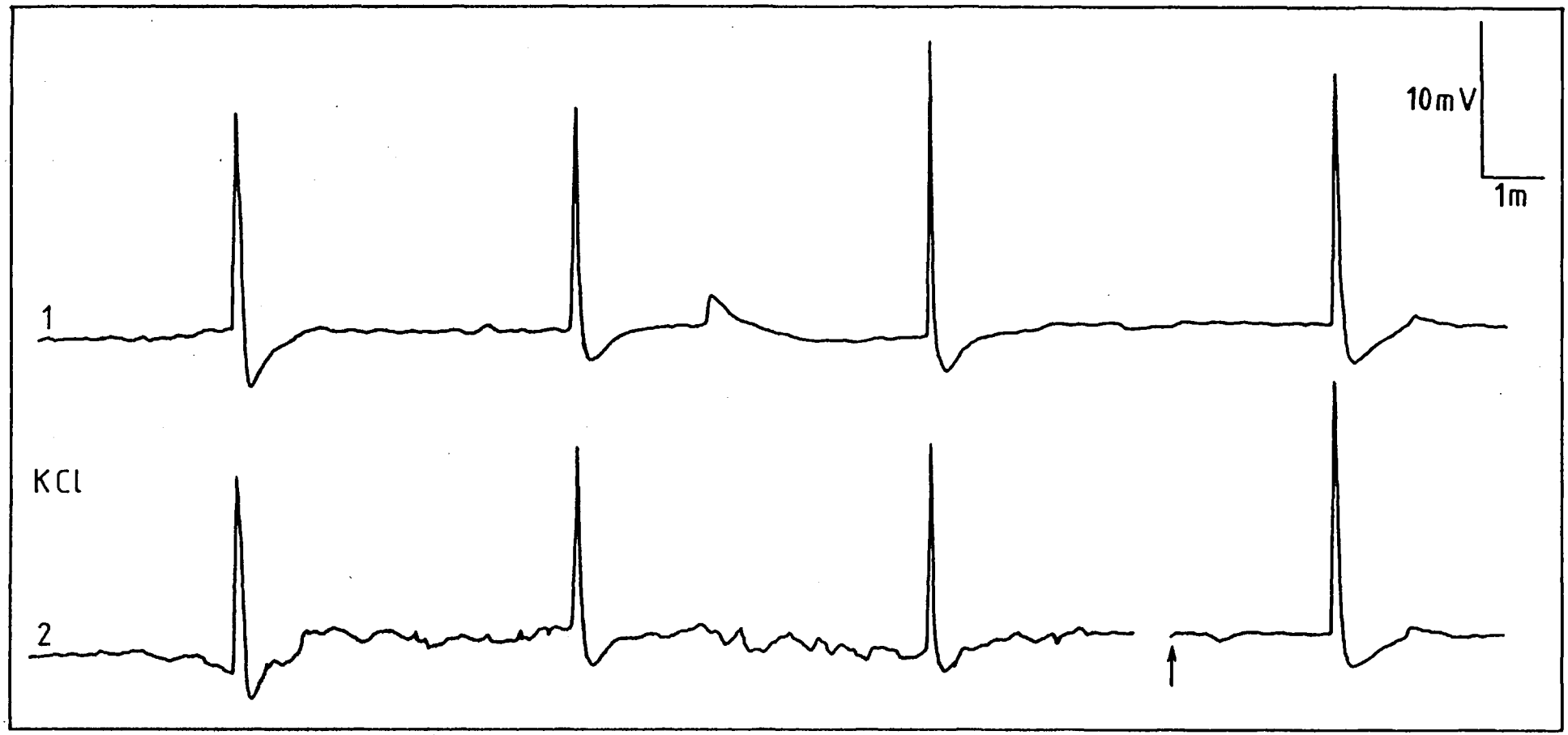


Fig. 3.28 An A.C. coupled recording showing the KCl-induced signals picked up (1) inside and (2) outside the vascular bundles by two tungsten electrodes. The stem was cut off just below the first leaf and the electrodes were inserted through the cut surface. At a point indicated by the arrow, electrode 2 was inserted into another vascular bundle.



Fig. 3.29 An A.C. coupled recording showing KCl-induced signals in the root and the stem. The soil was washed off the roots and the plant was kept with most of its root system, except for the upper part, submerged in the nutrient medium. Tungsten electrode 1 was placed inside the part of the root which was out of the medium and tungsten electrode 2 was placed on the stem. The reference electrode was in the medium. The stimulus was applied by adding KCl to the mediun.

in the stem. The closeness of the recording electrode in the root to the site of action potential production might account for the fact that the action potentials detected in the root were of opposite (negative) polarity to the ones in the shoot. The reason for the upper part of the root showing only one signal might be a short circuit developing between the recording and the reference electrodes due to KCl penetrating the tissue and the KCl-front reaching the recording electrode.

In order to check if the stem could be stimulated by KCl to produce signals, isolated internodal-sections (with leaves removed) were used. One end of the stem section was kept in a nutrient medium in contact with the reference electrode and the other end was in contact with a recording electrode. It was found that either the apical or the basal end of the stem-sections could be induced to produce a series of signals like the roots of a whole plant, although the concentration of KCl required to initiate the signals was very high (1 M).

Another series of experiments was carried out with a range of KCl concentrations in order to look at the effect of the strength of the stimulus on the signals produced. For these experiments, the soil was washed off the roots and the plant was kept with its roots submerged in the nutrient medium. The reference electrode was placed in the medium surrounding the roots and recording electrodes were placed on the aerial parts of the plant. The stimulus was applied either by adding an appropriate amount of saturated KCl solution to

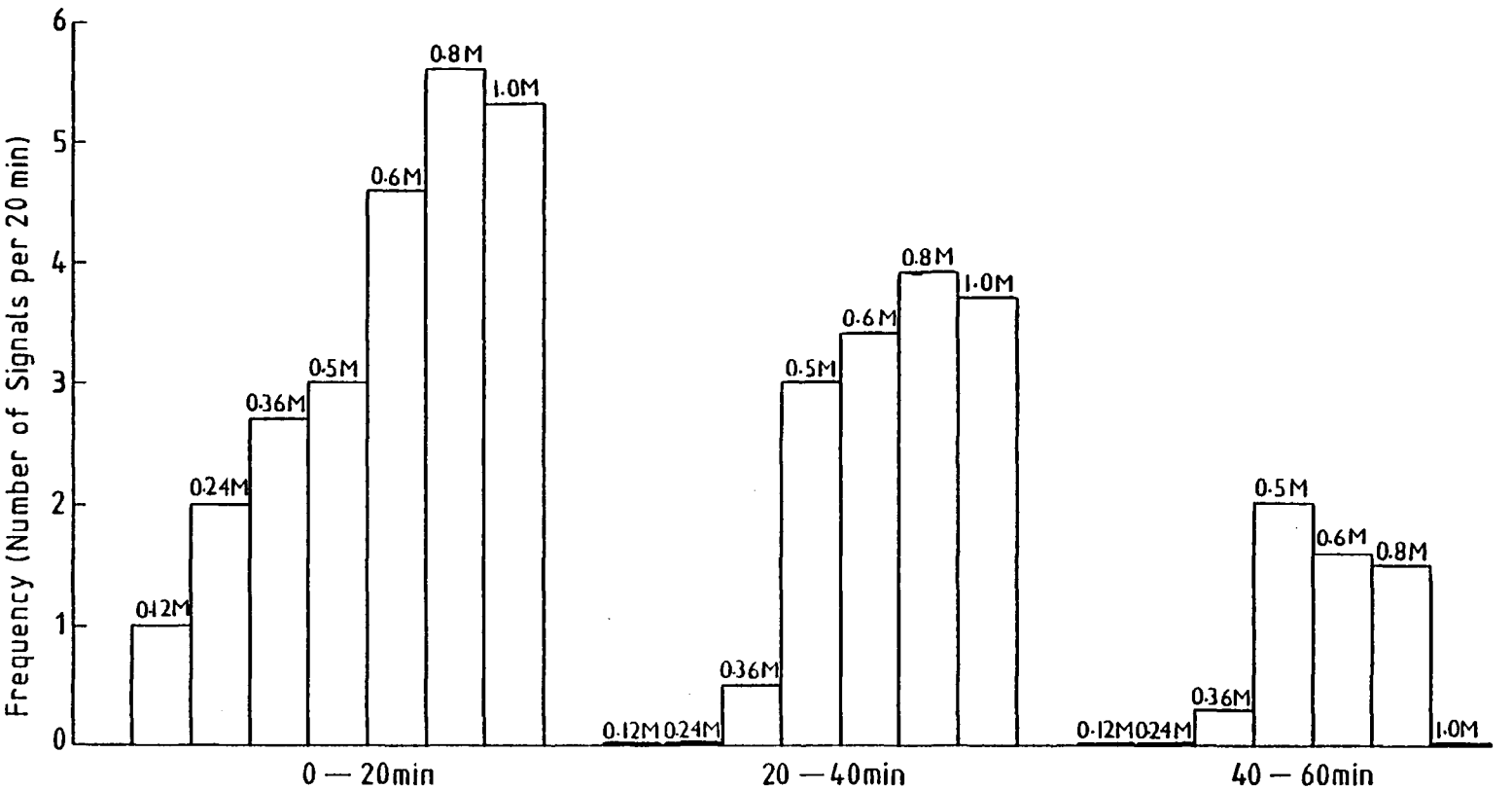


Fig. 3.30 Histograms showing the effect of KCl concentration on the number and frequency of signals induced in the roots. The results are means of at least 3 replicates. The signals taken into account for this figure and rest of the tables in this section were all larger than 10 mV. Since the latent period between the application of the stimulus and the first signal observed varied considerably, time 0 is taken as being the arrival of the first signal (this also applies to the rest of the results in this section).

achieve the desired final concentration or by replacing the nutrient medium with a KCl-containing medium of correct strength. It was found that the KCl concentration determined the number and frequency of the signals in a series, but had no effect on their amplitude. Figure 3.30 shows the relationship between the KCl concentration and the number (and frequency) of signals induced. The results shown in the figure suggest a threshold value and a saturation value of the stimulus. The figure also illustrates the drop in the frequency of the signals as the series progressed. The rather drastic drop in the frequency during the later stages (40-60 minutes) after 1 M KCl treatment could be due to damage or the death of the excitable tissues.

At this stage it can be said that KCl which is known to cause a reduction in cell membrane potential (Hope and Walker, 1961; Dunlop and Bowling, 1971) triggers off a series of action potentials. However, it was not possible to ascertain from the results whether KCl caused this depolarization directly (by K^+ entering the cells to reduce the negative charge inside) or indirectly (e.g. by reducing the water potential which may interfere with active ion transport).

3.3.4.2 Osmotica

In order to investigate whether the previously described KCl-stimulated signals were produced in response to its osmotic effect on the roots, some experiments were carried out using NaCl, $CaCl_2$ and mannitol as a stimulus.

The results show that NaCl, $CaCl_2$ and mannitol could stimulate signals in the roots which were detected synchronously on two

recording electrodes placed on the stem. Table 3.1 shows that NaCl was as effective as KCl in the short-term (in the first 20 minutes) but unlike KCl, increasing the NaCl concentration did not increase the number of signals any further. $CaCl_2$, although it induced some signals, was less effective than either KCl or NaCl. Similar experiments where mannitol was used to create a high osmoticum around the roots gave few signals, even at 0.8 M. Thus is can be said that the increase in osmotic pressure does induce action potentials in the roots but only in the short-term. The possible explanation for KCl being able to produce a larger number of signals might be that the cell membranes are more permeable to K^+ than the other ions tested and this maintains the cells depolarized due to its passive uptake. The effect of other ions and mannitol may be mainly one of osmotic shock and the action potentials die away when the osmotic equilibrium is reached. The mechanism by which KCl induces depolarization may be passive uptake of K^+ as well as increase in osmotic pressure.

Some experiments were conducted with mannitol and KCl together to check whether a combination of two different factors had an additive effect. The plant was kept with its roots in mannitol for about 20 minutes before the KCl stimulus was administered. The results given in Table 3.1 show that the concentrations of mannitol or KCl which would have had a little or no effect individually, when applied to the roots in combination had a marked stimulatory effect on the number and frequency of the signals produced suggesting that the stimuli are, in some way, additive.

Some more experiments were performed to look at the effect of removal of osmoticum on signal generation in the roots. Plants kept with their roots in 0.4 M mannitol for an hour or more could be induced to produce signals by replacing the mannitol with the normal

Table 3.1 Application or removal of osmoticum and the number of signals induced in the roots. The electrical measurement techniques were the same as described for KCl-induced signals in section 3.3.4.1.

Treatment	Number of signals from		
	0-20 min	20-40 min	40-60 min
KCl (0.12 M)	1	0	0
KCl (0.24 M)	2	0	0
KCl (0.5 M)	3	3	2
NaCl (0.24 M)	3	0	0
NaCl (0.5 M)	3	0	0
$CaCl_2$ (0.5 M)	1	0	0
Mannitol (0.2 M)	0	0	0
Mannitol (0.4 M)	1	0	0
Mannitol (0.8 M)	2	0	0
Mannitol (0.2 M) + KCl (0.12 M)	3.3	2.3	2.4
Mannitol (0.2 M) + KCl (0.24 M)	5.2	2.8	1
Replaced 0.4 M mannitol with normal nutrient medium	2	2.3	1.8
Watered a wilted plant	5	1	0

The results are means of at least 3 replicates.

nutrient medium. In the same way the roots of a wilted plant could also be stimulated to give signals by watering the dry soil of the pot. Thus, an application or removal of water stress, can both stimulate action potentials in the roots.

3.3.4.3 Anaerobiosis

In nature, at times, the soil in which the plants are growing gets water-logged due to flooding. This would limit the oxygen supply to the roots. It is well known that the cell membrane potential is maintained by ATP-driven pumps, and it might be predicted that reducing energy availability by limiting the oxygen supply could lead to lowering of its value. In order to check whether the depolarization produced in this way could initiate any action potentials, some experiments were performed where oxygen-deficient condition around the roots were simulated by bubbling nitrogen through the nutrient medium in which they were submerged. Table 3.2 shows that bubbling nitrogen, for up to 40 minutes, through the nutrient medium did not induce any signals in the roots.

Some further experiments were carried out to investigate the effect of combination of two factors (anaerobiosis + KCl) on signal generation. This was done by passing nitrogen through the nutrient medium containing roots of the plant for about 40 minutes and then applying a KCl stimulus. The combination had a synergistic effect on the number and the frequency of the signals produced, suggesting that the anaerobic environment does lower the membrane potential but it does not on its own lower it to the threshold value which would lead to action potentials in the roots.

Table 3.2 Anaerobic treatment and the number of signals induced in the roots. The electrical measurement techniques were the same as described for KCl-induced signals in section 3.3.4.1.

Treatment	Number of signals from		
	0-20 min	20-40 min	40-60 min
N_2	0	0	-
KCl (0.24 M)	2	0	0
N_2 + KCl (0.24 M)	5.6	4	3.7

The results are means of 4 replicates.

3.3.4.4 Auxins

IAA is known to depolarize the cell membrane potential (Goring et al., 1979), therefore, a range of IAA concentrations were applied to the roots to check if it induced any signals. The results in Table 3.3 show that IAA at a concentration as high as 10^{-4} M did not itself stimulate any signals in the roots nor did 2,4-D, at a concentration of up to 10^{-4} M.

In another series of experiments, combinations of IAA and KCl at various concentrations were tried to examine their combined effect on signal generation. This was done by keeping the roots of a plant in the IAA solution for about 20 minutes and then adding KCl to achieve the desired concentration. Table 3.3 clearly shows a synergistic effect of various combinations on the number of signals produced by the roots. IAA at a concentration as low as 10^{-12}M was just as effective as the higher concentrations (10^{-6} M). The most interesting fact which emerged from these experiments was that, after the IAA pretreatment, the latent period, between KCl stimulus and the first signal observed, was reduced to a few seconds (Figure 3.31). Also the signals were more uniform in the shape, and the decline in the size and frequency was very gradual. The fact that the latent period was reduced to a few seconds after IAA pretreatment, suggests a possible change in the permeability of the cells to K^+.

3.3.4.5 Gibberellic Acid (GA)

GA application to the roots did not induce any signals. GA was also tried in combination with KCl. The roots of a plant were treated with GA for about 2 hours before the KCl stimulus was applied. The results given in Table 3.4 show that the combination did not produce any more signals than the control (without GA pretreatment).

Table 3.3 Auxin treatment and the number of signals induced in the roots. The electrical measurement techniques were the same as described for KCl-induced signals in section 3.3.4.1.

Treatment	Number of signals from		
	0-20 min	20-40 min	40-60 min
IAA (10^{-12} M)	0	0	0
IAA (10^{-9} M)	0	0	0
IAA (10^{-6} M)	0	0	0
IAA (10^{-4} M)	0	0	0
2,4-D ($4.5\ 10^{-6}$ M)	0	0	0
2,4-D (10^{-4} M)	0	0	0
KCl (0.12 M)	1	0	0
KCl (0.24 M)	2	0	0
KCl (0.36 M)	2.7	0.5	0.3
IAA (10^{-12} M) + KCl (0.12 M)	1	1	1
IAA (10^{-12} M) + KCl (0.24 M)	4.4	5.6	1.6
IAA (10^{-12} M) + KCl (0.36 M)	7.2	5.3	4
IAA (10^{-9} M) + KCl (0.12 M)	1	1	-
IAA (10^{-9} M) + KCl (0.24 M)	5	3	3
IAA (10^{-6} M) + KCl (0.12 M)	1	1	-
IAA (10^{-6} M) + KCl (0.24 M)	5.3	3.7	2

The results are means of at least 2 replicates.

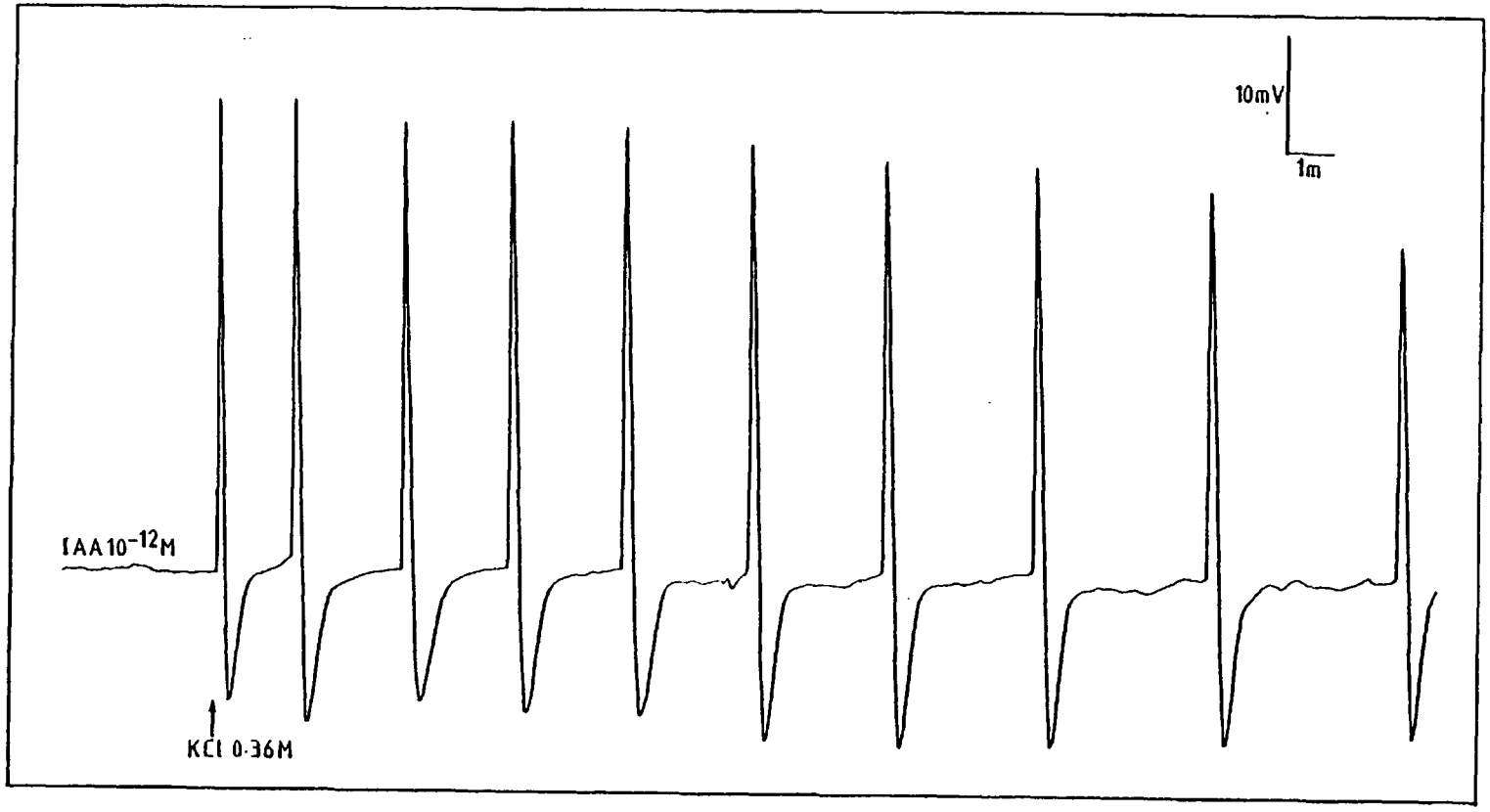


Fig. 3.31 An A.C. coupled recording showing the stimulation of KCl-induced signals in roots pretreated with 10^{-12} M IAA. The tungsten electrode was on a leaf petiole and the reference electrode in the medium around the roots.

3.3.4.6 Kinetin (FAP)

Kinetin when applied to the roots did not stimulate any signals. Kinetin which is supposed to affect cell permeability to K^+ ions (Ilan, 1971; Ilan et al., 1971), was also applied in combination with KCl. The plant was kept with its roots in a kinetin solution for 2 hours before the KCl stimulus was administered. Table 3.4 shows that the pretreatment with kinetin had no obvious effect on the number of signals produced by the roots as a result of the KCl stimulus, suggesting that kinetin does not have any major short-term effect in increasing membrane permeability to K^+ in this tissue.

3.3.4.7 Abscisic Acid (ABA)

Abscisic acid is also known to regulate ion (especially K^+) fluxes (Van Steveninck, 1972; Cram and Pitman, 1972). Therefore it was applied in combination with KCl. KCl stimulus was administered to the roots which had been kept in ABA for 2 hours. The results in Table 3.4 show that ABA did not induce any signals itself nor did pretreatment with it have any obvious effect on the number of signals generated by a KCl stimulus.

3.3.4.8 Acetylcholine (ACh)

Acetylcholine, which is also reported to affect the cell membrane potential and membrane permeability to ions (Jaffe, 1970), when applied to the roots did not induce any signals. ACh was also applied in combination with KCl. The roots of a plant were kept in ACh for about 20 minutes before the KCl stimulus was administered. The results in Table 3.4 show that ACh may have had a slight effect. The number of signals, induced by the KCl stimulus, had somewhat increased after ACh pretreatment, however the effect was nowhere

Table 3.4 Treatment with some growth regulators and the number of signals induced in the roots. The electrical measurement techniques were the same as described for KCl-induced signals in section 3.3.4.1.

Treatment	Number of signals from		
	0-20 min	20-40 min	40-60 min
KCl (0.24 M)	2	0	0
KCl (0.5 M)	3	3	2
GA (3.10^{-5} M)	0	0	0
GA (3.10^{-5} M) + KCl (0.24 M)	2	0	0
FAP (5.10^{-5} M)	0	0	0
FAP (5.10^{-5} M) + KCl (0.24 M)	2.5	0	0
ABA (5.10^{-4} M)	0	0	0
ABA (5.10^{-4} M) + KCl (0.24 M)	2	0	-
ABA (5.10^{-4} M) + KCl (0.5 M)	3	3	1
ACh (10^{-5} M)	0	0	0
ACh (10^{-5} M) + KCl (0.24 M)	2.5	1	0
ACh (5.10^{-3} M)	0	0	0
ACh (5.10^{-3} M) + KCl (0.24 M)	3	1	1

The results are means of at least 2 replicates.

near as great as the effect of IAA.

3.3.4.9 Ascorbic Acid (AA)

Ascorbic acid, which is a reducing agent and which was suspected to have electrical effects because it has been shown to induce leaf closure in *Mimosa* (Hesse et al., 1957), was used to stimulate the roots. AA when applied to the roots at a concentration of 10 mM did not induce any signals. However, AA, at a concentration of 100 mM (which is unphysiological), did induce a series of signals in the roots which were detected synchronously on two recording electrodes placed on the stem (Table 3.5). That this effect of AA was not due to its acidity was checked by using a nutrient medium which was adjusted to pH 2.8 (the pH of 100 mM AA was 2.8). AA, even at the lower concentration, when in combination with KCl had a stimulatory effect on the number of signals produced by the roots.

The signals induced by 100 mM ascorbic acid proved an excellent tool for studying the ion requirements of the roots for signal generation. A number of experiments were carried out to investigate the importance of external Ca^{2+}, Na^{+} and K^{+} ions in the production of action potentials. The plants to be used were taken out of the pots, the soil was washed off their roots and they were kept in the greenhouse for 2 days with their roots in an aerated nutrient medium which was deficient in one of the three cations. Before the start of an experiment the plant was kept in the same medium and the stimulus was administered by replacing this with a similar medium containing 100 mM AA. The results in the Table 3.5 show that Ca^{2+} deficiency did not affect the stimulation of signals by AA, Na^{+} deficiency had some effect, but the lack of K^{+} in the medium seriously affected the ability of the roots to produce signals. The plants kept in distilled

Table 3.5 Ascorbic acid treatment and the number of signals induced in the roots. The electrical measurement techniques were the same as described for KCl-induced signals in section 3.3.4.1.

Treatment	Number of signals from		
	0-20 min	20-40 min	40-60 min
KCl (0.24 M)	2	0	0
AA (10 mM)	0	0	0
AA (10 mM) + KCl (0.24 M)	6	2	-
Nutrient medium (pH 2.8)	0	0	0
AA (100 mM, pH 2.8)	4.6	2	2
AA (100 mM) + KCl (0.24 M)	7	5	-
AA (100 mM) in medium lacking Ca^{2+}	4	4.3	1.7
AA (100 mM) in medium lacking Na^{+}	2	0	0
AA (100 mM) in medium lacking K^{+}	0	0	0.5*
AA (100 mM) in distilled water	2*	0	0

The results are means of at least 2 replicates.

*The size of the signals was very small (2-4 mV).

water produced a few, but very small signals. Thus, these experiments clearly show the importance of K^+ ions for the production of action potentials in the roots of a plant.

3.3.4.10 Cyanide

Some experiments were conducted to check if KCN could inhibit the signal-producing-ability (e.g. after KCl stimulus) of the roots. The results, however, showed that KCN treatment itself induced signals. The KCN (10 mM) treatment of the roots of a plant produced a series of small signals which were detected synchronously on two recording electrodes placed on the aerial parts of the plants. This suggests that KCN induced a depolarization which is large enough to initiate action potentials.

3.4 Transmembrane Potential Measurements in Root Cells

Microelectrode measurements of intracellular potentials, under different conditions, were undertaken in order to confirm and relate them to the results obtained in section 3.3.4. The intracellular potentials of cortical cells in the root segments of marrow seedlings were measured with respect to the bathing medium using D.C. coupled amplifiers. The results shown in this section are chart records from single experiments, although numerous measurements yielded qualitatively similar results.

3.4.1 Preliminary Experiments

Early work on intracellular potential measurement showed that the membrane potentials of cells in the root segments obtained from 3-day-old etiolated seedling was no different from that obtained from a 4-5-week-old plant grown in the light. Therefore, subsequent work was carried out with root segments excised from 3-day-old seedlings. Further measurements were carried out to check if the cells in different regions in the root showed any differences in their membrane potentials. Figure 3.32A shows that there were no major differences in the membrane potentials of cells in the regions at various distances from the root tip. For the remaining measurements, the microelectrode was inserted in a region 3 to 5 mm behind the root tip. It can be seen from the figure that once the electrode tip was inside a cell the reading was quite stable.

3.4.2 Effect of KCl Treatment

KCl applied at a concentration of 0.12 M induced a depolarization of the cell membrane potential. Further increase in KCl concentration (0.24 M) led to a bigger depolarization, sometimes

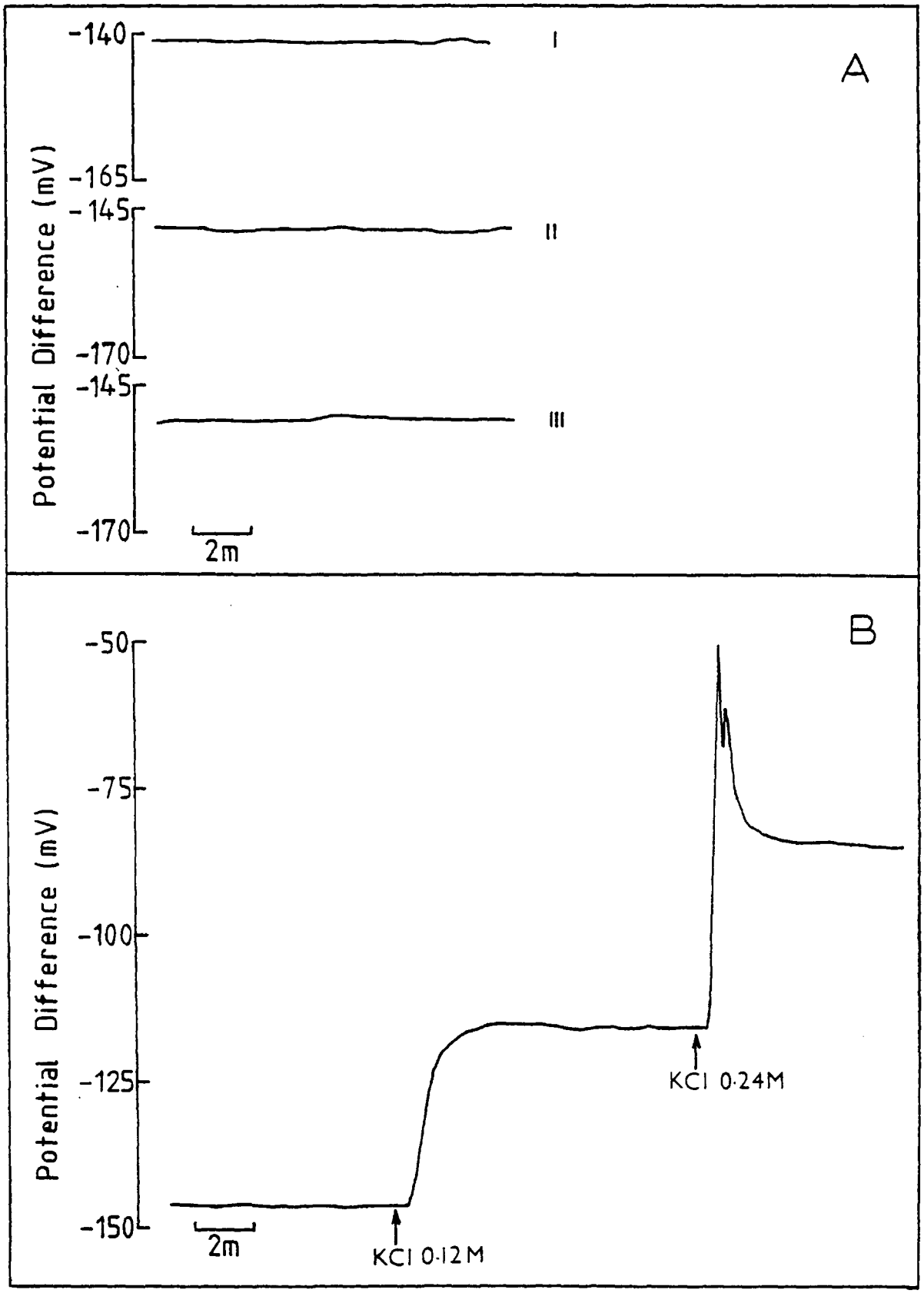


Fig. 3.32 (A) Membrane potential of cortical cells of the root in a region (I) 2 mm, (II) 5 mm and (III) 8 mm from the tip. (B) Effect of KCl on the membrane potential of a cortical cell in the root.

accompanied by an action potential (Figure 3.32B). Thus, these results confirm the suggestion, made in section 3.3.4.1, that the higher the concentration of KCl in the bathing medium the higher is the resultant depolarization.

3.4.3 Effect of Increase in Osmotic Pressure

When mannitol was added to the bathing medium to create a high osmotic pressure around the root cells, it caused a partial depolarization. Figure 3.33A demonstrates the effect of such a treatment on the cell membrane potential. Increased osmotic pressure led to a drop in the membrane potential and the higher the pressure the larger was the resultant depolarization. Often the depolarization was accompanied by action potentials. These results are in line with those obtained from the whole plants described in section 3.3.4.2, and suggests that the action potentials were due to a depolarization resulting from a reduction in the water potential.

3.4.4 Effect of Anaerobiosis

In order to create anaerobic condition, nitrogen rather than air was bubbled through the bathing medium containing the root segment. This treatment induced a slow depolarization of the cell membrane potential by about 40 mV (Figure 3.33B). No action potentials were ever observed as a result of anaerobic treatment. Reaeration of the medium induced a repolarization of the membrane potential to its previous level. The results indicate that a depolarization of 40 mV is not big enough to induce any action potentials. Again there is a confirmation of the results observed with surface electrode measurements on the whole plants described in section 3.3.4.3.

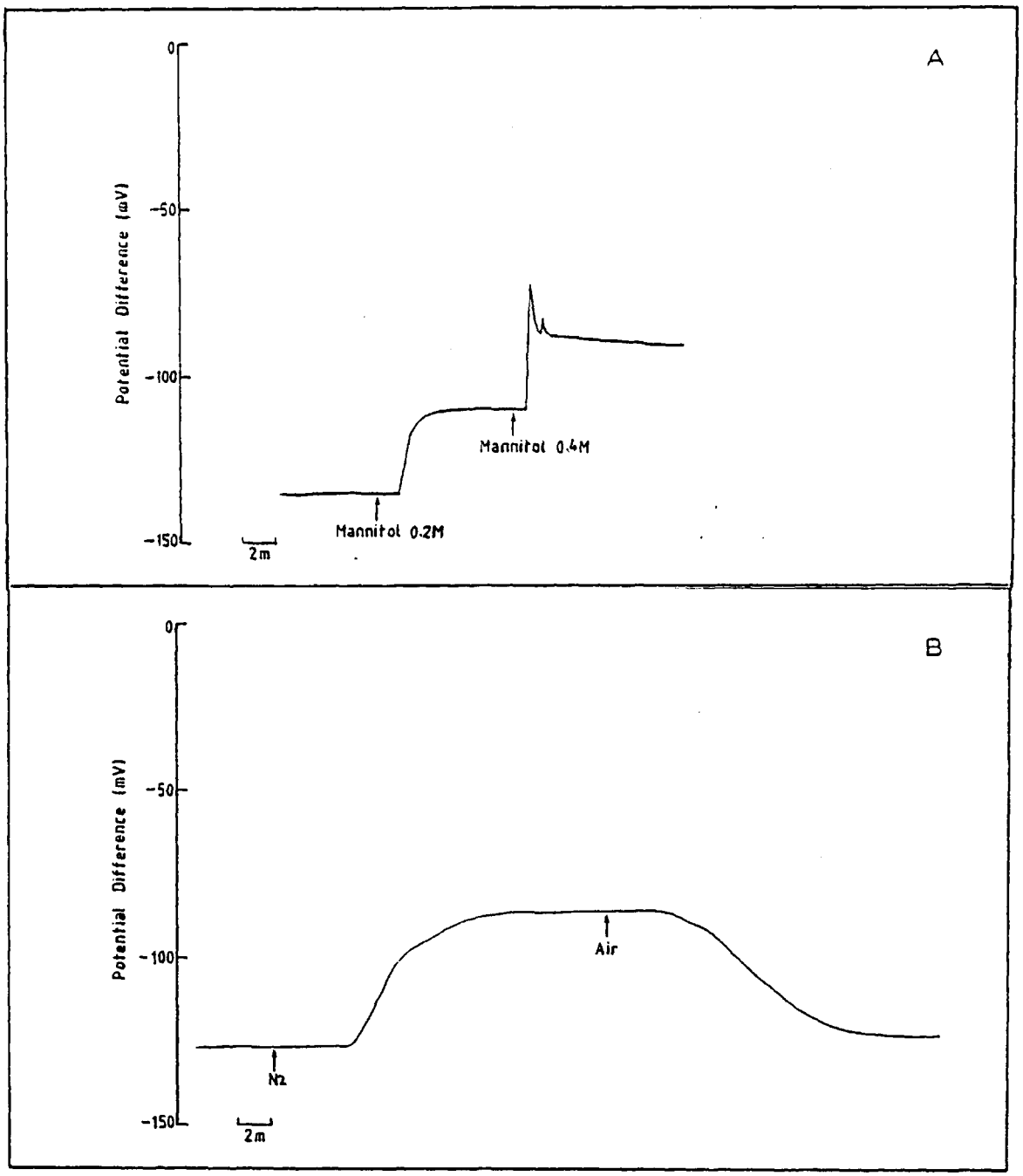


Fig. 3.33 (A) Effect of osmotic pressure on the membrane potential of a cortical cell in the root. (B) Effect of anaerobic condition on the membrane potential of a cortical cell in the root.

3.4.5 Effect of IAA Treatment

IAA at a concentration of up to 10^{-7} M did not affect the cell membrane potential in any way, but higher concentrations (10^{-6} and 10^{-5} M) did induce a depolarization of about 20 mV in a few minutes (Figure 3.34). Further experiments were conducted to examine the effect of IAA pretreatment on the permeability of the cells to K^+. The root segments used for these experiments were incubated in IAA for about 20 minutes before the membrane potential measurements began. The results illustrated in Figure 3.35 clearly show that KCl induced a bigger depolarization after the root segments were pretreated with IAA. The suggestion is that IAA, at as low as 10^{-12} M concentration, increased the permeability of cells to K^+, thus, confirming the results obtained with whole plants given in section 3.3.4.4.

3.4.6 Effect of Ascorbic Acid Treatment

Ascorbic acid induced a drop in the cell membrane potential. The extent of depolarization depended on the concentration of ascorbic acid in the medium. Higher concentrations of ascorbic acid (100 mM) induced action potentials. These results are in line with those, described in section 3.3.4.9, observed with surface electrode measurements on the whole plants.

3.4.7 Effect of KCN Treatment

KCN at a concentration of 3 mM induced a slow depolarization of about 65 mV, but the membrane potential repolarized back to some extent although not to the original value. The repolarization process was accompanied by some small action potentials. Increasing the KCN concentration to 10 mM in the medium led to a further

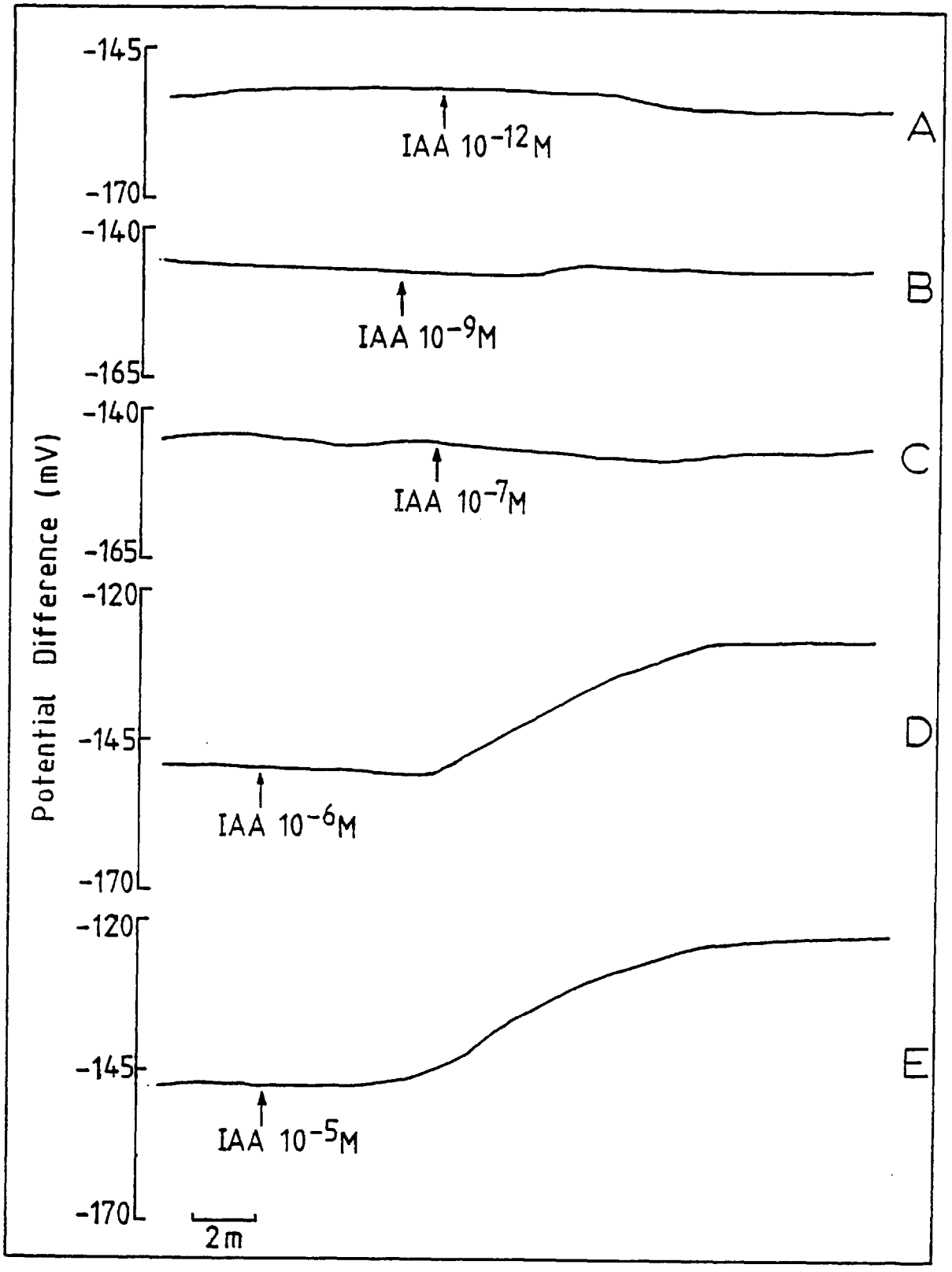


Fig. 3.34 Effect of a range of IAA concentrations on the membrane potential of cortical cells in the root.

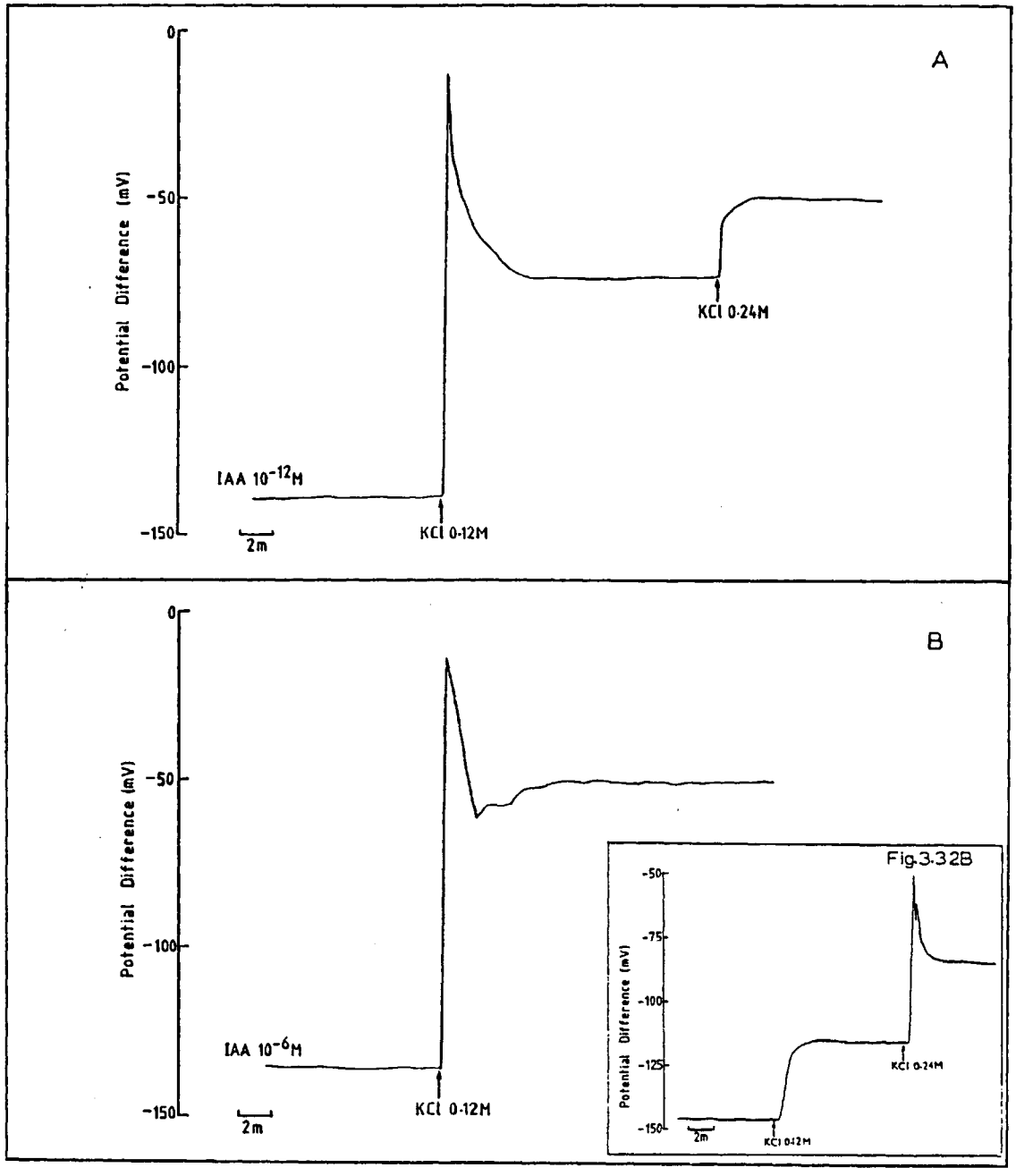


Fig. 3.35 Effect of KCl on the membrane potential of a cortical cell in the root pretreated with IAA (A) 10^{-12} M and (B) 10^{-6} M. Figure 3.32B is repeated for comparison.

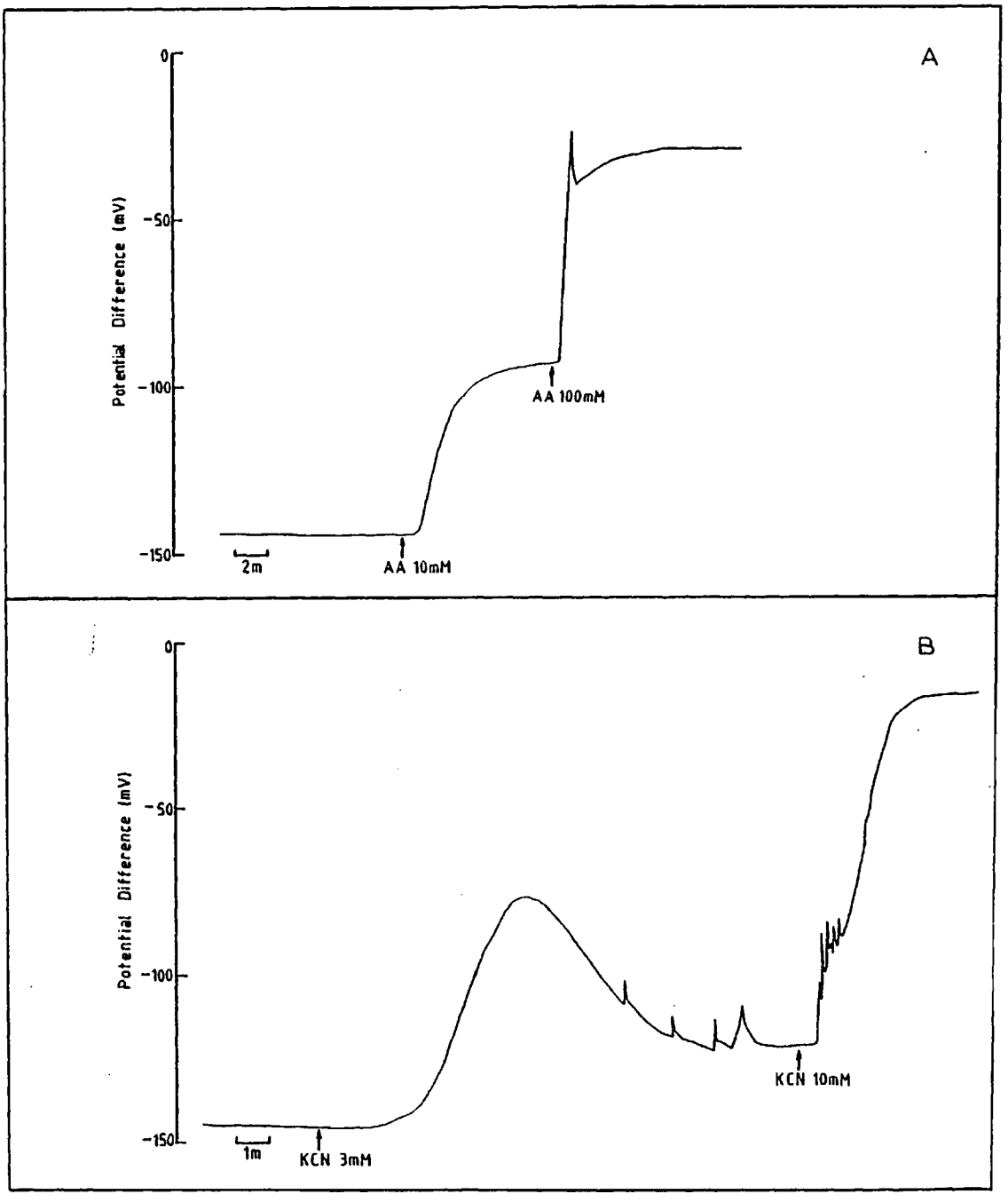


Fig. 3.36 (A) Effect of ascorbic acid on the membrane potential of a cortical cell in the root. (B) Effect of KCN on the membrane potential of a cortical cell in the root.

depolarization of about 100 mV from which it did not recover. Again a few more small action potentials were observed during the depolarization. These results confirm those, given in section 3.3.4.10, observed with whole plants.

3.5 The Independence of CO_2 Exchange from Action Potentials

Following some Russian work (Gunar and Sinyukhin, 1963) suggesting a link between electrical activity and the control of photosynthesis and respiration, some experiments were conducted with pumpkin plants, in order to discover whether the stimulated large action potentials had any effect on the rate of CO_2 exchange of a leaf away from the site of stimulus. KCl treatment of the roots and thermal injury to a neighbouring leaf were the stimuli used. Although KCl-induced signals in the root were found to be of a non-propagating nature and therefore should not be regarded as a means of communication, the treatment was nevertheless tried to check if it had any effect. The rate of respiration or photosynthesis of a leaf were measured with an infrared gas analyser and the electrical activity was recorded from the petiole of the leaf with a tungsten electrode connected to a high input impedance amplifier.

3.5.1 KCl Stimulus

KCl treatment of the roots induced a series of signals, which could be detected on the leaf petiole (Figure 3.37), but there was no obvious short term effect on the rate of respiration. However, there was a slow steady decline in the rate of respiration (about 10% in 60 minutes) but this could have been due to damage to the plant by the KCl treatment.

Figure 3.38 shows that the KCl stimulus had little or no effect on the rate of photosynthesis.

3.5.2 Heat Stimulus

Heat injury to a neighbouring leaf induced many signals which were picked up in the petiole of the leaf being used for

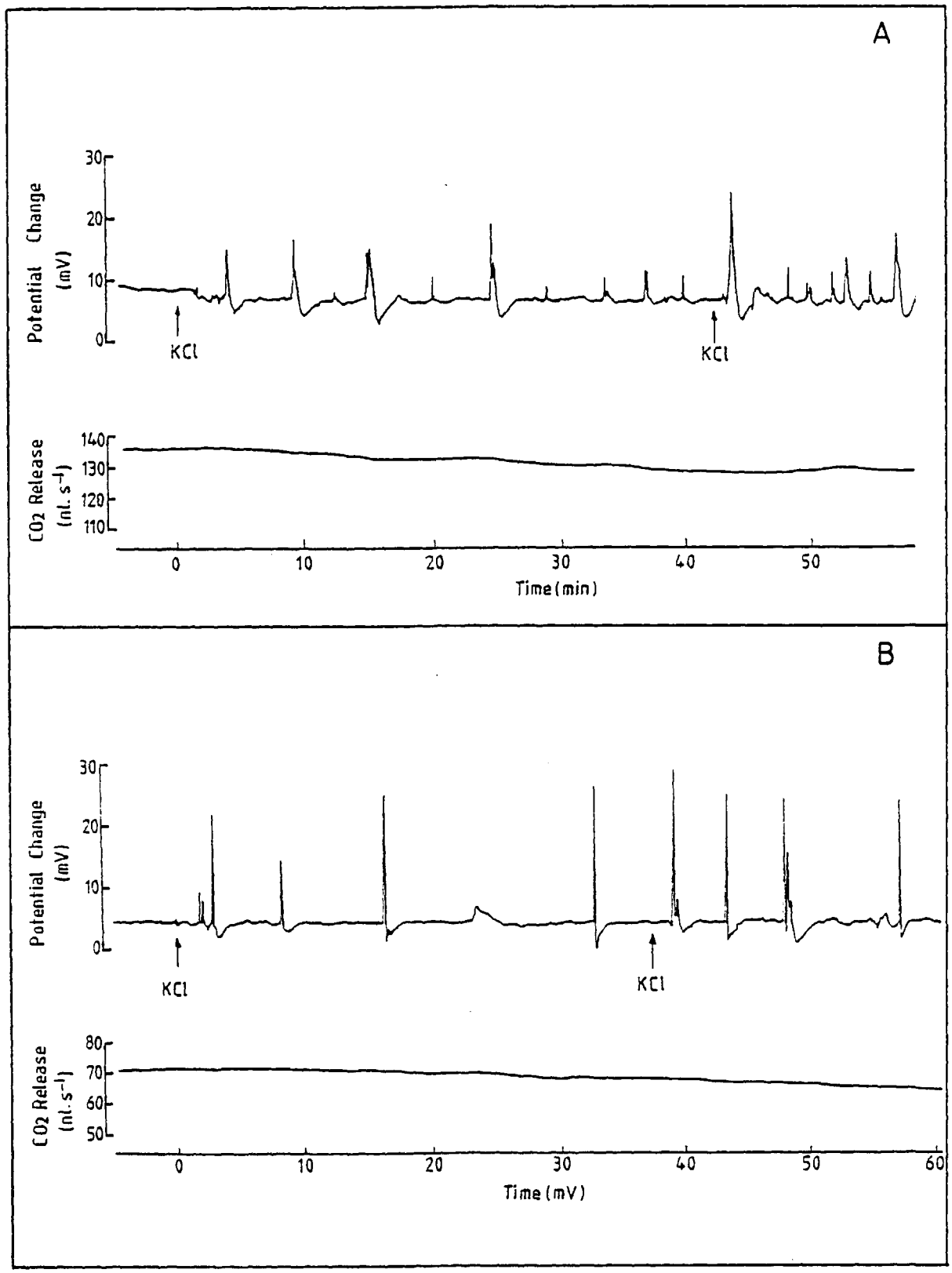


Fig. 3.37 Electrical activity and leaf respiration. (A) the upper trace is electrical activity recorded in the petiole following KCl application to the roots. The lower trace is a simultaneous measurement of the respiration of the leaf (area 238 cm^2). (B) A similar experiment with a smaller leaf (area 112 cm^2).

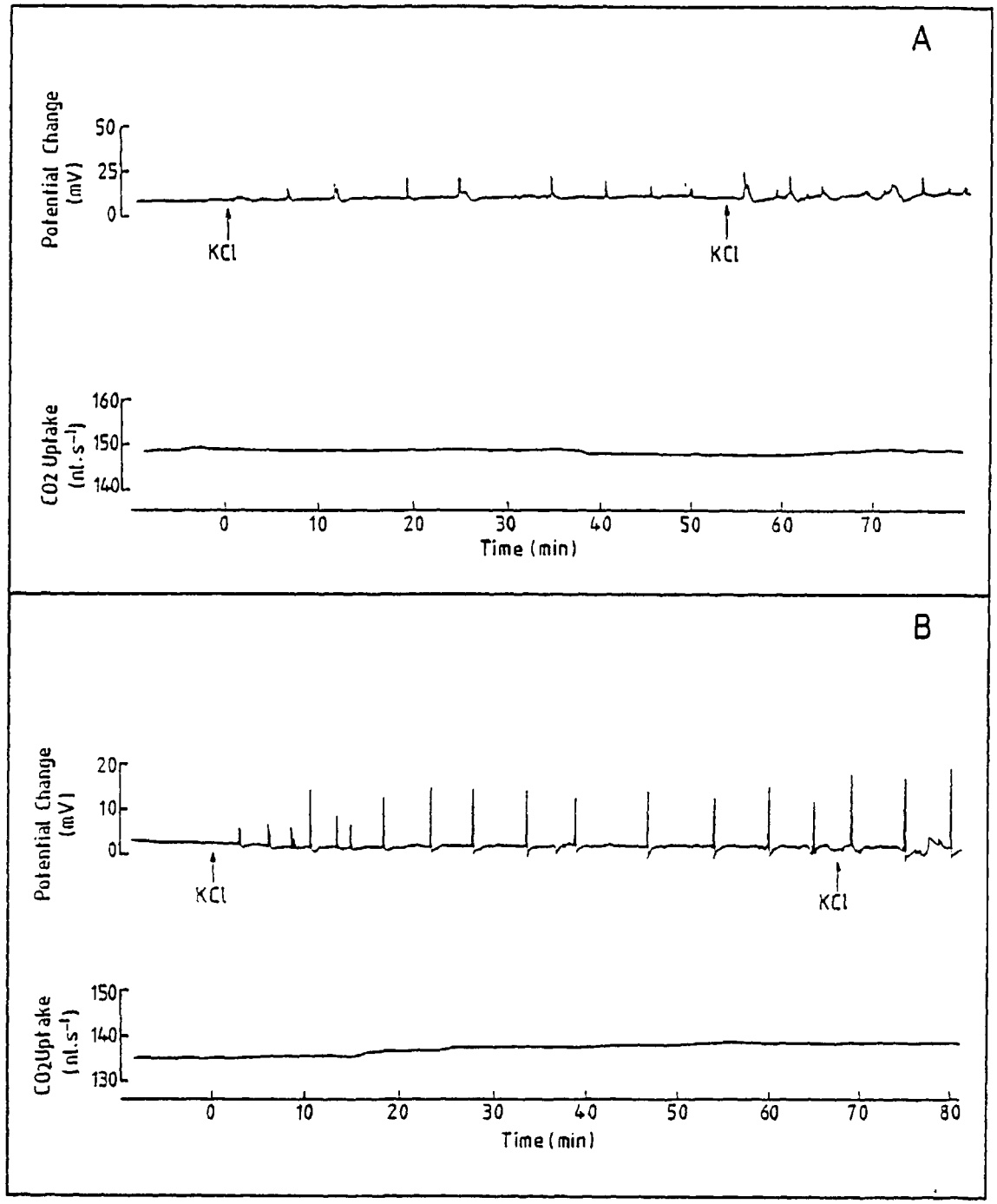


Fig. 3.38 Electrical activity and photosynthesis. (A) The upper trace is electrical activity recorded in the petiole following KCl application to the roots. The lower trace is a simultaneous measurement of the photosynthesis of the leaf (area 202 cm^2). (B) A similar experiment with another leaf (area 162 cm^2).

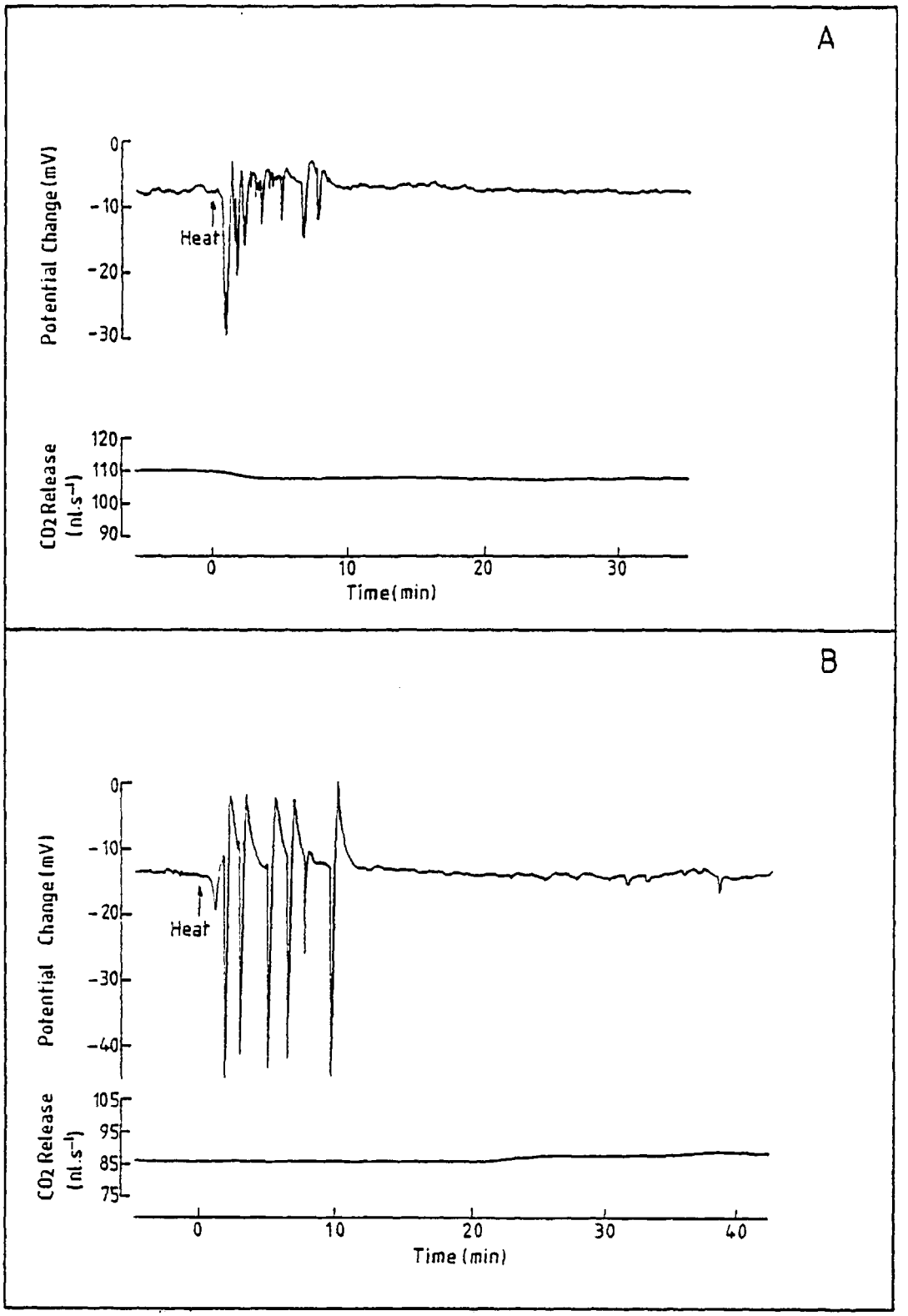


Fig. 3.39 Heat-induced action potentials and leaf respiration. (A) The upper trace is the electrical activity detected in the petiole and the lower trace is a simultaneous measurement of the respiration of the leaf (area 170 cm^2) when a neighbouring older leaf was heated. (B) A similar experiment with another leaf (area 156 cm^2).

respiratory measurements. Figure 3.39 shows that there was no consistent change in the rate of respiration after the arrival of heat induced signals.

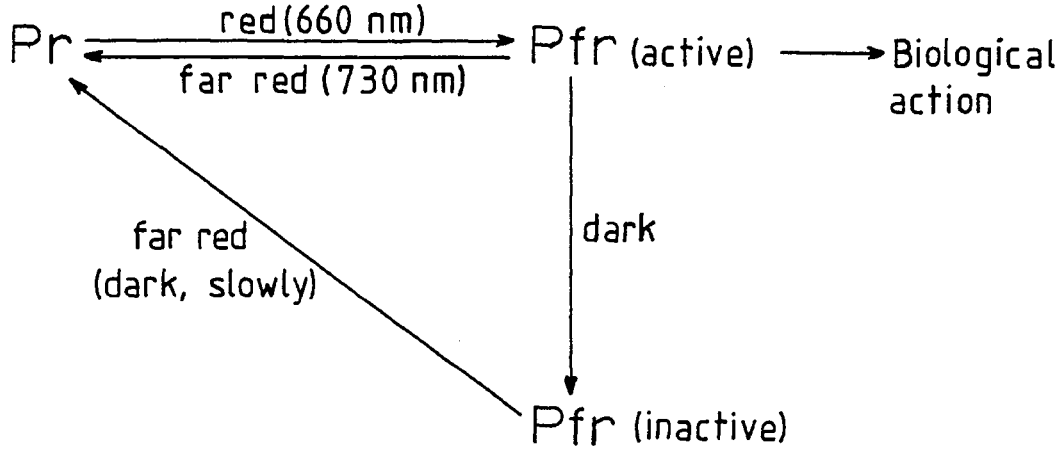
4. DISCUSSION

4 DISCUSSION

The results presented in this thesis clearly show the occurrence of electrical activity in many higher plant systems.

4.1 Phytochrome-mediated Electrical Potential Changes

The growth, development and movement of plants are strongly influenced by light energy which not only nourishes the plant through photosynthesis but also acts as a trigger which alters the pathways of metabolism to enable this to take place. The pigment phytochrome perceives low-intensity light and induces a chain of reactions finally leading to a number of visible morphogenetic responses (e.g. germination, de-etiolation, flowering etc.). At least part of this chain appears to involve a change in the electrical potentials across cell membranes. Phytochrome is a blue-green biliprotein readily soluble under alkaline conditions and bearing as a chromophore one or more bilitriene moieties closely related to the chromophores of the algal pigments phycocyanin and allophycocyanin (Siegelman et al., 1966). It is known that phytochrome exists in two photoconvertible forms: Pr, absorbing maximally at 660 nm, and Pfr, absorbing at 730 nm. The form produced by red irradiation is responsible for subsequent developmental events. Thus:



The mechanism of phytochrome action is one of the major questions in plant biology. Among the more well known mechanisms suggested are (a) gene repression and derepression, (b) enzyme activation or inactivation, (c) control of binding, release, or metabolism of hormones which then control the metabolic pathways in various ways, and (d) control of membrane properties. At first, the idea that phytochrome exerts its effects through gene activation (Mohr, 1966) was most popular, possibly because the phytochrome concept developed in an era when the importance of DNA and its role in metabolism was becoming widely studied. However, the popularity of this idea, as the universal mode of phytochrome action, did not last long as some very rapid phytochrome-mediated responses were discovered, e.g. chloroplast movement in *Mougeotia* (Haupt, 1965) and the nyctinastic movements of leguminous leaflets (Fondeville et al., 1966). These processes, because of their rapidity and insensitivity to inhibitors of RNA and protein synthesis, could not be explained on the basis of gene activation. Hendricks and Borthwick (1967) postulated that phytochrome is a controlling factor in membrane permeability, and that both the rapid and slow responses are consequences of this primary effect on the membrane. The discovery of phytochrome-mediated electrical potential changes at the root tips (Tanada, 1968a, b; Jaffe, 1968; Racusen and Miller, 1972) further added to the list of rapid phytochrome-phenomena. Thus it is clear that the initial reactions must involve rapid events beginning with molecular changes in the phytochrome holochrome initiated by red or far red light and therefore, it is very necessary to obtain as much knowledge as possible of the earliest events following the stimulation.

Photoreversibility (R-FR mutual reversibility) is considered

as the hallmark of phytochrome and is generally taken as evidence for phytochrome-participation in plant responses. The electrical potential changes in etiolated wheat coleoptiles, in the present study, which are red-far red reversible, suggests that phytochrome initiates this phenomenon. Red light induced a positive change in the potential at the wheat coleoptile tip and far red had the reverse effect. The response is a rapid one as the potential changes begin within 10 seconds of the onset of red or far red exposure. The results also indicate that under the conditions used, a two-minute exposure to red light was enough to transform the physiologically active Pr into Pfr in order to produce maximum electrical responses. The fact that the first exposure to red induced a positive change in potential, but far red without the preceding red was ineffective, supports the general belief that most of the phytochrome in the etiolated seedlings is in the Pr form which first has to be converted into the Pfr form before far red effects can be observed. Other work with intracellular electrodes is consistent with the data presented in this thesis for extracellular surface potential changes. Newman and Sullivan (1976) reported a hyperpolarization of the transmembrane potentials by red light in oat coleoptile cells. Rapid phytochrome-mediated transmembrane potential changes have also been observed in the motor cells of the nyctinastic plant *Samanea*, which precede leaf movement and may participate in its mechanism (Racusen and Satter, 1975). Tanada (1978) detected a far red-reversible increase in translocation of fluorescein following exposure to red light in etiolated mung bean hypocotyls and believes it to be due to generation of a positive electrostatic charge in the plasmamembrane by some action of phytochrome on the membrane molecules.

It is a well known fact that most bioelectrical potentials develop due to ion movements through membranes and therefore the phytochrome-mediated potential changes might be attributed to an alteration of membrane permeability. Confirmation for this view comes from the work of Jaffe and Galston (1967), who detected changes in membrane permeability in *Albizzia* motor cells during phytochrome-controlled nyctinastic sleep movements. Weisenseel and Smeibidl (1973) reported a phytochrome-induced change in water permeability in *Mougeotia*. The rapidity of the potential changes induced by phytochrome, in the present investigation, favours the idea that phytochrome is associated with membranes. However, it is an open question whether phytochrome modulates directly one or more membrane functions because it is an integral part of the membrane, or it (Pfr) first binds with a receptor in the membrane as an initial step in its function. Plasmamembrane-localization of phytochrome was suggested on the basis of studies on phytochrome-controlled chloroplast movement in *Mougeotia* (Haupt, 1965). Further investigations, using microbeams of polarized red and far red light, revealed that Pr and Pfr are oriented in the membrane, but in different planes (Haupt, 1970). If the phytochrome were structurally a part of the plasmalemma, as light altered its own molecular conformation or configuration, it in turn could alter the configuration of the membranes, perhaps changing permeability characteristics and inducing a localized electrochemical gradient, manifested as bioelectrical potential. On the other hand, physiological studies and extraction of phytochrome from etiolated seedlings led to the concept of phytochrome binding to receptors on cytoplasmic membranes (Marme et al., 1973; Rubinstein et al., 1969). Boisard et al. (1974) proposed a model for the binding properties of phytochrome, based on molecular interaction

at the membrane. They speculated that the functional changes in the membranes, which arise due to molecular interaction, could lead to change in permeability, rapid electrical potential changes and finally to the long-term photomorphogenetic responses.

The results illustrated in Figure 3.4 show that the absence of roots did not affect the ability of the seedling to show the phytochrome response. Also roots on their own did not show any R-FR reversible potential changes. It is possible that the potential changes induced at the root tips were too small to be detected by the technique used. The phytochrome-induced potential changes in the root tips reported by Jaffe (1968) were very small (1 mV). The results obtained by exposing different areas of the coleoptile show that the magnitude and the polarity of phytochrome-induced potential changes remained the same irrespective of the fact that whole, upper part, or lower part of the coleoptile was illuminated. A similar phytochrome-mediated phenomenon was observed by Newman and Briggs (1972) in oat coleoptiles. They found that when an area of the coleoptile in a region 5 mm from the tip was illuminated, an electrode at 2 mm from the tip (in the dark) recorded a potential change of a similar amplitude as another electrode placed on the illuminated region. The electrical response observed in etiolated wheat seedlings can be explained because the phytochrome is present in fairly high amounts in all etiolated seedlings (Briggs and Siegelman, 1965). In general, phytochrome-levels are highest in meristematic or recently meristematic tissue of both root and shoot, and substantial synthesis occurs during development. At the tissue level, phytochrome is highest just behind the coleoptile tip, decreasing down the coleoptile, and increasing again at the node. It might be reasonable to assume that the tip which contains the greatest

amount of phytochrome would evoke the largest potential changes. However, since decapitation does not have an immediate effect on phytochrome-induced potential changes, this seems unlikely. The results of Figure 3.3, which suggest that all parts of the coleoptile are equally perceptive, can perhaps best be explained by assuming that the externally measurable potential changes between the tip and the base of the coleoptile reflect an integration of non-uniform changes in cellular membrane potentials resulting in cellular polarization (in other words, the red and far red lights make the cells behave like batteries along the longitudinal axis), and the polar response produced in one region may spread throughout the coleoptile. The system must involve movement of some ions as in the case of leaflet closure in *Albizzia*, but at the moment it is difficult to specify which, and further investigation into this is needed.

The coleoptiles completely lost their ability to respond electrically to R-FR treatments after about an hour of decapitation. Since the photo- and gravity-induced potential changes were shown to be mediated by IAA redistribution (Johnsson, 1967) and as the IAA concentration is higher in the coleoptile tip, it was thought that phytochrome-mediated electrical changes might also be transmitted by IAA and the loss of the IAA source resulted in a diminished response. However, substitution of IAA for the tip did not maintain the R-FR reversible electrical response, indicating that some other factor must be involved. Furthermore, the sheer rapidity of the electrical response tends to discount the possibility that the phytochrome-mediated potential changes depend on the movement of IAA in a way analogous to the development of photo- and geo-electric potentials.

Also the fact that illumination of the lower part of the coleoptile could induce a potential change at the tip and that the electrical response was not lost immediately after tip removal, is evidence against a direct mediation of IAA in the phytochrome-induced potential changes. Satter et al. (1972a) found that changes in endogenous auxin do not control leaflet angle of *Albizzia* plant in the light, neither is it involved in other phytochrome-regulated process, since it does not alter phytochrome-control of leaf movement or potassium flux. Lurssen (1976), on the other hand, observed a counter-action of IAA-induced H^+-excretion by the phytochrome in *Avena* coleoptiles and speculated that the counter-action may occur at the level of primary action of both auxin and phytochrome in the plasmalemma. However, since Tanada (1968a) showed that IAA is needed to observe the photoreversibility of the phytochrome-mediated root tip adhesion phenomenon, it is quite possible that IAA along with some other factor(s) may interact with the phytochrome system at some point and may be involved in some of the responses.

Electrical potential changes are not the only rapid phytochrome-mediated responses. Jaffe (1970) observed a rapid phytochrome-induced increase in the acetylcholine content of the secondary root tips of mung bean, and showed that ACh mimicked some of the red light effects, like H^+-efflux, reducing the formation of secondary roots and causing the adhesion of root tips to a negatively charged glass surface. He proposed that phytochrome governs the morphogenetic phenomena by its control of ACh. Evidence both for (Hartmann, 1971; Kandeler, 1972; Saunders and McClure, 1973) and against (Kasemir and Mohr, 1972; Satter et al., 1972b; Tanada, 1972) this proposal has been presented and therefore, at the moment it can be said that ACh might mediate phytochrome-action in some systems but

not in all. Many other rapid phytochrome-mediated systems have been reported recently, but their exact relationship with the rapid electrical changes is not yet clear. White and Pike (1974) showed rapid (1 min) changes in ATP level induced by the phytochrome system in etiolated bean buds and speculated that the formation of Pfr may alter the activity of ATPases (conformational change of the membrane-bound phytochrome could affect the structure and hence the activity of other membrane-bound proteins) which in turn could modulate the activity of ATP-dependent ion pump systems. On the other hand, it is also possible that the increase in ATPase activity is an indirect result of other phytochrome-mediated potential changes. A phytochrome-mediated change even in the passive permeability of membranes would change the membrane potential, and therefore, the voltage gradient across any proteins within it. This voltage gradient is of immense magnitude (about 10^7 $V.m^{-1}$) since the membrane is very thin, and therefore, a change in it would be expected to have a considerable effect on the conformation of membrane-bound enzymes (e.g. ATPase), and hence their activity. Graber et al. (1978) have already shown the stimulating effect of externally applied potentials on the ATPase activity of chloroplasts. Changes in the ATP pool brought about in this way may influence ion pumps and the protein-synthesizing machinery. Other membrane-bound enzymes might also be affected in this way. Further rapid phytochrome-mediated changes include NADPH levels (Manabe and Furuya, 1973), nitrate reductase activity (Johnson, 1976), NAD kinase activity (Tezuka and Yamamoto, 1974). There might be several other similar systems which are rapidly influenced by phytochrome and the rapid changes in the activity of the crucial enzymes and coenzymes may have wide ranging effects on cellular metabolism. It would be interesting to

investigate the exact causal relationship between the electrical changes and the biochemical changes.

The results described in this thesis provide further support to various other reports suggesting that electrical changes are one of the primary events induced by the phytochrome action, however, there is a lot more to be learnt about the exact mechanism, the ions involved and the significance of the polarity of the electrical changes, before any simple model can be given to explain the system. The technique used for electrical measurements in this investigation has its limitations and it is possible that the phytochrome-induced potential changes are a lot bigger than observed. Grahm and Hertz (1962) reported that the gravity-induced potential changes using vibrating electrodes were 4 times larger than when the conventional contact electrodes were used. The use of vibrating electrodes might therefore be better for measuring potential changes from different regions of the coleoptile and hence help to understand the location and mechanism of the electrical response. In the end it can be said that the long-term phytochrome-controlled morphogenetic responses which involve gene activation are either a secondary manifestation of the initial changes in the membrane properties, or phytochrome has a dual action, one involved in rapid non-morphogenetic responses, the other involved in processes controlling growth.

4.2 Spontaneous Electrical Activity in Light-grown Plants

In general, action potentials have been thought to be associated with sensory and communication system in animals and a few so-called "sensitive plants". However, the results presented in this thesis and several other reports emphasize that action potentials

are also associated with, and seem to be a general feature of a wide variety of higher plants lacking in any type of motor activity.

Action potentials can be recorded either intracellularly or extracellularly. The intracellular measurements are made by inserting a fine recording microelectrode inside a cell, with a reference electrode placed in the bathing medium surrounding the cell. The extracellular action potential is measured with both electrodes placed at some distance from one another, each making contact via a salt bridge with a large number of cells. In the present investigation, surface located Ag/AgCl and tungsten electrodes implanted into the tissue were used for recording the signals in plants. However, it should be noted that the tungsten electrodes, although sharpened to a fine point, were not as fine as a glass microelectrode, and might have damaged the cells through which the tip penetrated. Therefore, the recordings obtained from them should not be regarded as intracellular. There is evidence that these electrodes do behave as extracellular electrodes. A comparison of the recordings from tungsten and Ag/AgCl electrodes of heat-induced signals and action potentials from the lobes of Venus fly-trap showed that they both recorded the signals in negative polarity as would be recorded with an extracellular electrode. Therefore, it would be fair to say that a tungsten electrode not implanted too deeply in the stem or petiole could effectively be in contact with many cortical cells.

By taking proper precautions to eliminate noise, 4 different kinds of non-evoked electrical activities were observed in the shoots of 4-week-old marrow plants.

4.2.1 Repetitive Spiking

Bursts of repetitive spikes with a well-defined shape and rather constant amplitude were found to be produced at irregular intervals by the plant tissue. The signals in most of the trains showed action-potential-characteristics, i.e. a relatively fast rise and a slow return. The general regularity of these signals within a train and their resemblance to animal nerve impulses suggest that they might possibly play a role either in signalling or in regulating some physiological process. The regularity of signals also indicates that they arise from a common origin such as a single cell or a group of cells firing in synchrony. Similar trains of repetitive signals have been reported by Pickard (1972) in *Ipomoea*, *Pisum* and *Xanthium*. Karlsson (1972) also reported repetitive bursts of pulses in India-rubber tree and believes them to be generated due to the activity of a group of cells on the basis of electrode arrangement he used.

The amplitude of the signals, although almost constant within a train, varied quite a lot from train to train. Trains with signals of amplitude as small as 0.25 mV and as large as 4 mV were found in different recordings. In comparison, the signal amplitude reported by either Pickard (1972) or Karlsson (1972) were quite small (the largest being about 0.25 mV). To some extent the amplitude of signals might depend on the electrical path through which they have to pass. The shapes of the action potentials in most of the trains reported in this study for marrow plants were quite comparable to those of the signals reported by Pickard (1972) in *Ipomoea*. It is not possible to make a direct comparison with the shapes of signals from the India-rubber tree, because band pass filters were used in the measuring circuit (Karlsson, 1972).

The decrease in amplitude and deceleration in the firing rate, observed in some cases, near the end of a train might be due to fatigue. However, since it did not happen in all cases, other factors might also be involved which control the termination of a train.

These action potentials were never found to show true propagation. The evidence for this comes from the fact that the trains of action potentials, whenever picked up by two electrodes placed some distance apart, were detected in absolute synchrony. Therefore, their conduction from their site of origin must be very largely electrotonic. The passive electrotonic conduction follows the core-conductor theory which means that a tissue system, consisting of cells which are coupled through plasmodesmatal connections, acts like a leaky (or poorly insulated) coaxial cable. The cell walls being the outer conductor, the membranes the insulator (leaky) and the cytoplasm the inner conductor. An electrical event, such as an action potential can be conducted through the system electrotonically over a considerable distance and can be recorded by extracellular or intracellular electrodes. The size of the electrical signal recorded depending upon the distance from the site of occurrence, number of cells involved in the generation of the signal, and the resistance of the electrical path (e.g. good or poor plasmodesmatal connections).

However, if true propagation occurred within the tissue over only a very short distance (a few cells) the time delay would not have been detectable, so a limited degree of true intercellular propagation remains possible. In this investigation, apart from the special case of heat-induced signals propagating in the phloem, virtually all the signals recorded were of positive polarity,

regardless of whether the electrode was external or internal to the plant body. This was initially surprising, because an action potential, resulting from a cell's depolarization (due perhaps to a rapid release of Cl^-) should cause an external electrode to go negative. However, this would only be the case when the electrode was very close to the firing cell. When many cells are connected together to form a symplast, the more positive internal charge developed within the firing cells will be transmitted electrotonically to the remainder of the symplast via the plasmodesmata. This, in turn will cause the passive exchange across their membranes, of ions to which they are freely permeable (perhaps a release of K^+ into the apoplast). Thus an action potential would be expected to cause a negative-going signal in the apoplast in the immediate vicinity of the firing cells, but a positive-going signal would emnate from the cells which are away from the firing cells, the amplitude of which would decline with increasing distance from the site of origin. The evidence presented in the thesis indicates that even the spontaneous repetitive, low level signals have an electrotonic range of the order of centimetres. The vast majority of signals would therefore have a "distant" origin, and would be expected to be of positive polarity.

The unusual shape of some signals in a train might be due to their conduction through complex pathways of different electrical properties within the tissue, which would distort their appearance. The apparent overlapping of two signals, which was observed on rare occasions, might be due to the combined effect of independent action potentials in two separate cells or groups of cells.

That the trains of action potentials observed were biological in origin and not electrical noise was deduced from the fact that when a 10 M resistor was connected in place of the plant, the

activity disappeared.

Thus, it seems that many higher plants produce trains of repetitive bioelectrical signals intermittently as a part of their normal activity. Similar action potentials form a basis for communication in animal nervous systems. The most remarkable aspect of comparison between plant and animal nerve action potentials is not their differences but that two types of cells so far removed on the biological scale should have active membrane properties with so many features in common. It seems likely that the action potential wave which is a characteristic of all types of cells, has merely been adapted in the nerve cell to the function of communication. In the plant kingdom also there are some species, e.g. *Dionaea* (Sibaoka, 1966; Benolken and Jacobson, 1970), *Drosera* (Williams and Pickard, 1972 a, b; Williams and Spanswick, 1972) and *Mimosa* (Sibaoka, 1966) etc., which use action potentials to mediate the movement of some of their organs by a mechanism analogous to true nervous conduction.

4.2.2 "Irregular" Fluctuations

At times another kind of electrical activity, with relatively larger (1-10 mV), irregular signals of either polarity, was observed. It is possible that some of the signals might be due to injury to the cells when a tungsten electrode was used, however, the evidence that most of them are genuine non-evoked, natural electrical activity comes from the facts that they were also detected by surface located Ag/AgCl electrodes (which have little injurious effect) and that they could be detected a long time after the tungsten electrodes had been implanted in the tissue. As the signals were only picked up by one of the two recording electrodes placed as close as 1 cm, it seems that they might be due to the activity of cells in the

immediate vicinity of the electrodes and are not conducted over long distances, electrotonically or otherwise. Since most signals showed a fast rise and a slow return to the base-line, it is possible that they were action potentials, however, they might equally be a result of some other effect. Pickard (1972) has reported similar "irregular" signals (though they were smaller in size), and speculated that these signals could either be action potentials or incidental manifestation of activities like pinocytosis or the extrusion of vesicles.

4.2.3 Complex Low Level Activity

It is possible that the complex electrical activity might be a result of a superposition of fluctuations from several cells firing in an asynchronous manner. The fact that some parts of the activity recorded by two electrodes placed about 2 cm apart showed similarity suggests that this type of electrical activity can be conducted electrotonically over distances of this order. The "quiet periods" observed between trains of repetitive spikes etc. probably consist of this very low level complex activity which can only be recorded at the high sensitivities on the recorder. Since the complex electrical activity has been shown to be conducted over distances of the order of centimetres in the tissue, the extremely low level of activity sometimes observed might be due to the fact that it was occurring in regions some distance from the electrode.

4.2.4 "Solitary" Signals Recorded at Two Different Points Simultaneously

The simultaneous detection of very rare, large (8-35 mV) signals at two widely distant points indicates their electrotonic

conduction and possible remote origin. They show the action-potential-characteristics of a relatively fast rise and a slow return to the base-line. These signals had many features in common with those detected in the stem after stimulation of the roots by KCl, e.g. relatively greater size, simultaneous detection at two widely distant points on aerial parts and positive polarity. As with the KCl-induced signals, their sheer magnitude suggests that they cannot be the result of the firing of a single, isolated cell but are the result of many cells firing in near synchrony. However, if these signals are really a result of the synchronous firing by a large number of cells in the root, or some other part, it is hard to explain what factors stimulate them to fire action potentials simultaneously in this way.

4.2.5 Spontaneous Electrical Activity and the Effect of Light Conditions and Hormonal Factors

A physiological interpretation of the observed bioelectrical activity (especially the repetitive action potentials) is clearly needed. The results presented in this thesis indicate that there is no direct correlation between the observed electrical activity in the shoot of marrow plants and the most important parameter essential for the function of the plant, the light. Single leaves or the whole plant kept either in the dark or the light for varying periods, showed no obvious changes in their electrical activity. Most of the recordings showed complex electrical activity and some showed activity with "irregular" signals. These results are in agreement with the reports by Pickard (1972) and Karlsson (1972) who did not observe any major effect of changes in the light intensity on the electrical activity of the plant. The results are an indirect

indication that the observed electrical activity in the petioles is not correlated with the rate of phloem translocation because the changes in the light conditions which are known to affect phloem activity had no observable effect on the electrical activity of the petiole. The results from the experiment, where a leaf was treated with kinetin, show that kinetin treatment which induces the movement of nutrients and metabolites from neighbouring regions had no effect on the electrical activity, again lending support to the suggestion that the observed electrical activity in the petioles is not associated with the phloem transport. Furthermore, when the electrodes were deliberately implanted in the phloem, no especially large signals were recorded as would need to be the case if the latest electroosmotic theory of phloem transport were to be correct (Spanner, 1979).

The results of further work carried out using IAA, 2,4-D, ACh and ABA suggest that the observed electrical activity is not directly affected by the application of these important hormonal factors although they have been shown to have a considerable effect on the cell membrane potential and permeability. Since the repetitive spiking occurred more frequently in the younger growing parts of the plant, it was thought that the higher IAA level in them was in some way responsible for the signals. However, application of IAA or 2,4-D to the leaf blade or the petiole near the electrode had no effect on the electrical activity of the petiole. The hormones did not stimulate any repetitive spiking. Thus, the suggestion is that auxins despite having a significant effect on the transmembrane potential (Cleland et al., 1977; Goring et al., 1979) and membrane permeability (Ilan, 1973), do not have any noticeable short-term effect on action potentials. However, the long-term

effects of auxin (due to its effect on growth and interaction with other substances etc.) cannot be discounted on the basis of the short-term experiments performed in the present investigation. ACh and ABA, when applied to the leaf also had no obvious effect on the electrical activity of the petiole. Thus, these substances, which have also been reported to affect cell membrane permeability and potentials (Jaffe, 1970; Cram and Pitman, 1972; Jones and Mansfield, 1970; Kriedemann et al., 1972), appear to have little or no effect on the mechanism which is responsible for generating action potentials.

4.2.6 Speculations

The significance of the action potential-like activity still remains obscure. However, one of the striking facts which emerged from the present study is that the repetitive spiking was almost always found to occur in the young growing regions of the shoot, indicating the association of this type of signal with the growth. There are a few more systems in higher plants where the electrical activity seems to be associated with the growing parts of the plant. Bean roots show spontaneous electrical fluctuations with the highest amplitude in the region of cell elongation (Scott, 1957). The action potentials detected by Pickard (1971) after the mechanical stimulation of pea epicotyls might well be as a result of growth responses in this region. The repetitive signals observed in *Ipomoea* (Pickard, 1972) were also from growing organs as most of the recordings were taken from the cotyledons of young plants. Similar spontaneous recurrent action potentials have been observed in *Acetabularia* during regeneration of cap. Thirty hours before cap generation begins, in enucleated stalk segments of *Acetabularia* which have been

morphologically depolarized by a light treatment, 100-sec-long current pulses (closely resembling action potentials) begin to enter one end and it is this end which finally regenerates (Novak and Bentrup, 1972). This seems to be as a result of growth rather than cap formation. Goodwin and Pateromichelakis (1979) showed that the Ca^{2+}, and Mg^{2+} ionophores, A23187, reversibly inhibits the formation of apical structures (whorls and caps) but does not arrest longitudinal growth in regenerating *Acetabularia*, and this growth still correlates with the spontaneous recurring action potentials which arise at the apex.

It is possible that the action potentials associated with the growing regions are a direct result of localised membrane damage, due to its stretching and breaking, and are produced in a way analogous to the formation of heat-induced action potentials. On the other hand, they might equally well be a result of changes in the osmotic pressure which normally occur during the growth of a cell. As will be discussed later this type of action potential might be involved in osmoregulation and maintenance of turgor pressure essential for growth.

Long-term continuous measurements of plant bioelectrical activity at various stages of growth are needed to carry out further investigation on the association between the two.

4.3 Heat-induced Electrical Activity

Propagating "waves of depolarization" or action potentials are typical of nerve and muscle cells in animals, where they carry information, concerning changes in the environment and mediate the responses. Among the various types of electrical activity in non-motile higher plants, the activity induced as a result of heat or

other kinds of injury bears the closest resemblance to the nerve impulses of animals and the electrical activity in *Mimosa*. On the basis of their experiments, Ricca (1916), Umrath (1927), and Snow (1924) concluded that, as a result of severe injury, *Mimosa* secretes a wound substance, whose movement through the petiole and stem is accompanied by an electrical disturbance. This compound is capable of transmission through dead tissue. Houwink (1935) established that there are two types of electrical excitation in *Mimosa*, depending on the strength of the stimulus. Weak stimulation (e.g. a drop of cold water or vibration) elicited an action potential. An action potential is a transient fall in the transmembrane potential of a cell. The fall in potential is relatively more rapid than the subsequent recovery and occurs as a result of temporary change in the permeability of the membrane. Very little is known about the ionic basis of the action potentials in higher plants, but large algal cells, such as *Nitella* (Hope and Walker, 1975) and *Acetabularia* (Gradmann et al., 1973), have been reported to show an enhanced Cl^- efflux during the depolarization phase of their action potential. The action potential propagates like a wave along the length of a cell and reaching the end excites another cell to produce an action potential. In *Mimosa* small phloem and protoxylem cells are involved in the transmission of action potentials (Sibaoka, 1962). A strong stimulation (e.g. cutting or burning) evoked variation potentials as well as action potentials. The variation potentials are due to a stimulating substance, set free at the wound, transported in the vessels, and which slowly depolarizes the cells locally as it moves. Both stimuli could bring about the folding of the leaflets. However the variation potential unlike an action potential could be transmitted through a dead or physiologically inactive zone in the stem by

diffusion of the wound hormone, and could evoke an action potential beyond the inactive segment. As a result of his observations in *Mimosa* and non-sensitive *Vitis discolor*, Houwink (1935) speculated that action potentials and variation potentials could be a general feature of all the higher plants, and therefore, the only special feature of *Mimosa* seems to be that it has gone one step further in adapting the action potentials to mediate the closure of its leaflets. In addition to *Mimosa*, the action potentials in the insectivorous plants *Drosera* and *Dionaea* also have a profound functional importance in the transmission of information from the sensory part to the motor parts. It is interesting that in all these cases the signals are limited to specific regions e.g. the phloem and the protoxylem in *Mimosa*, the tentacles of *Drosera* and the trap-lobes of *Dionaea*, and this may represent the beginning of development of a primitive differentiated nervous system.

In the present investigation, a heat stimulus elicited one or more, large (10-35 mV), propagating signals in marrow plants. These signals, induced by heat injury to a single leaf were of different shapes and sizes and their duration varied from 5 to 60 seconds. However, they bore characteristics of action potentials since they, almost always, showed a fast rise and slow return to the base-line. Similar heat-induced action potentials have been observed by Sinyukhin and Gorchakov (1966a) in several plants including *Cucurbita pepo*, *Helianthus annus*, *Phaseolus multiflorus*, and *Fagopyrum sagittaeum*. Furthermore, Sinyukhin and Gorchakov (1966b) reported that a heat stimulus to a pumpkin plant usually elicited a large number of action potentials whose amplitude ranged from 8 to 11 mV. They claim that a single heat stimulus usually induces two series (or groups) of action potentials and each series contains about 2.7

action potentials. It is surprising that in a previous publication (Gunar and Sinyukhin, 1963) with one common co-author, no mention was made that a heat stimulus could induce more than one action potential. However, the results of the present investigation indicate no grouping of action potentials.

The most clearly distinguishable characteristic of the action potentials induced by heat is their propagating nature. The rate of basipetal propagation of the action potentials, which arose as a result of heat stimulus to the leaf, down the petiole was about 6-10 $mm.s^{-1}$. Large action potentials propagating down a petiole could cross the node and spread acropetally as well as basipetally into the stem and the neighbouring leaves. Although there are some reports which suggest that action potentials do not cross a node (Houwink, 1938 quoted by Pickard, 1973), it seems that they might be of a different nature as they were cold-induced action potentials and their rate of propagation was relatively slower (2 $mm.s^{-1}$). My observation, that action potentials may cross a node, is further supported by the reports of Van Sambeek and Pickard (1976a) who observed that heat-induced action potentials often propagated out of the damaged leaf and into the neighbouring undamaged leaves. The rate of acropetal propagation of the action potentials in the stem, which arose due to a heat stimulus to the roots, was 3-5 $mm.s^{-1}$. Thus, in general, the rate of basipetal propagation of action potentials is higher than the rate of acropetal propagation. The rates of acropetal and basipetal propagation of action potentials reported by Gunar and Sinyukhin (1963) and Sinyukhin and Gorchakov (1966b) for pumpkin plants were similar to those observed in the present investigation. These rates of propagation for the heat-induced action potentials in non-motile higher plants are close to those in the

vascular bundles of *Mimosa*. Bose (1906) was the first to show clearly that the vascular bundles in plants perform two functions; i.e. (I) the transport of substances and (II) the spread of excitation from some organs to others. Sinyukhin and Gorchakov (1968) supported the idea that the propagation of action potentials in the non-motile higher plants also occurs in the vascular bundles by dissecting away the surrounding tissue for a length of 10-20 mm and showing that the signals could still be transmitted. They further showed that the action potentials could not pass through a region poisoned with dinitrophenol. It is possible that the small specialized cells in the vascular bundles are involved in the propagation of the action potentials, as in the case of *Mimosa* (Sibaoka, 1962). Some evidence for this comes from the work of Sinyukhin, 1964 (quoted by Pickard, 1973), who carried out some intracellular measurements on *Cucurbita pepo* and *Heracleum sibricum* and found that the small parenchymatous cells of the phloem and also the parenchymatous cells in the vicinity of protoxylem, had large resting potentials and were excitable.

The results of the present investigation and the reports discussed so far seem to suggest that action potentials are the only type of electrical activity which results due to a heat treatment. However, Van Sambeek and Pickard (1976a) reported that in *Lycopersicon*, the heat treatment induced a variation potential (about 25 mV) which might be accompanied by small action potentials. The variation potentials propagated from the site of injury and spread throughout the shoot. They also showed that slight squeezing, cutting or ice treatment of the petiole induced an action potential whereas severely crushing the petiole produced a variation potential. The implication was that the variation potential is produced only as

a result of a strong stimulus. In the present study, however, the most severe type of injury (burning a leaf with a lighted match) did not induce a variation potential in most cases, but action potentials were always elicited. Figure 3.22 is the only example in the present work where there is some indication of a small variation potential. It is possible that the variation potentials are produced in some but not in all types of plants, or if they are produced in all plants they do not spread very far from the site of injury. For example, Van Sambeek and Pickard (1976a) observed that variation potentials in *Lycopersicon*, initiated by heating, diminished rapidly when moving down the stem against the normal direction of water flow. This type of detrimental effect of acropetal transpiration on the basipetally moving variation potential may be more in some plants than the others. Van Sambeek and Pickard (1976b) further demonstrated that an extract of *Lycopersicon* leaf tissue when applied to the base of an excised *Lycopersicon* leaf caused a variation potential to spread through the leaf. They also labelled the extract with P^{32} and showed that the arrival of the variation potential coincided with that of the radioactivity, and suggested that the spread of the variation potential depends on the spread of the action factor (a wound hormone which they have termed as Ricca's factor) in the extract via the transpiration stream. Using microelectrodes, Cheeseman and Pickard (1977) showed that the extract containing Ricca's factor depolarized the membrane potential of three types of cell (mesophyll, midrib parenchyma and midrib epidermis) in *Lycopersicon* leaves and they believe that the depolarization was due to a direct action of Ricca's factor on the cell membrane. However, the basipetal propagation (at a rate of about 10 $cm.s^{-1}$ in the petiole) of the variation potential, reported by Van Sambeek and

Pickard (1976a), would be hard to explain if it occurred only as a result of hormonal movement in the transpiration stream which is acropetal in direction, unless a reversed flow is produced as a result of heat-damage to the leaf. A more definitive proof, that the transmission of a variation potential occurs due to the local depolarization of the cells as Ricca's factor moves, could have been obtained by checking that a variation potential was detected beyond a dead or physiologically inactive zone.

The heat-induced action potentials, in the present investigation, detected by a tungsten electrode implanted into the cortical tissue, or a surface Ag/AgCl electrode, were of negative polarity and showed the characteristics of true propagating action potentials as detected extracellularly. The action and variation potentials observed by Van Sambeek and Pickard (1976a) also had a negative polarity when picked up by the extracellular Ag/AgCl electrodes. When the action potential propagating in the vascular bundle reaches just beneath the recording electrode, the extracellular fluid becomes negative as the inside of the cells depolarizes and hence, the recording electrode placed in cortex or at the surface registers the action potential as negative.

The formation and propagation of an action potential must require some energy. There is some evidence that free radical reactions may provide the energy for the process. Kalinin et al., (1970) reported an intensive formation of free radicals during the passage of an action potential (induced by heat or KCl stimulus) along a vascular bundle in the leaves of fodder beet. Furthermore, they found that free radical centres were more abundant in the small cells of the phloem, thus confirming the contemporary view that this tissue is responsible for the transmission of action potentials in

plants. However, they did not mention where the reference electrode was positioned, how the propagation was measured, and whether or not the propagation differed with two kinds of stimuli. Opritov and Kalinin (1970) reported that the basipetal propagation of an action potential in the conducting bundles, induced by heat treatment (65°C), 0.5 M KCl and 0.1 M 2,4,5-T, was associated with increased ATPase activity, and accelerated acropetal transport of P^{32}, Ca^{45}, and glycine-C^{14}. Although, insufficient data were presented to show what the channels of translocation were, they implied that translocation occurred in the phloem. They did not comment on the fact that calcium is not appreciably mobile in the phloem. Opritov (1978) reported that stimulation of the roots of a pumpkin plant by KCl or chilled water induced an acropetally propagating action potential which was associated with basipetal transport of C^{14} from the leaves and speculated that the excitation wave may be a factor enabling a rapid response in the form of increased phloem loading following changes in environmental conditions. The biggest objection to their theory is that the KCl-induced action potentials have been regarded as similar to heat-induced signals but the results presented in this thesis show that they are fundamentally different. Those induced by heat show true propagation but those induced by KCl do not. However, if true for the heat-induced action potentials, the significance of this type of translocation may possibly be in the supply of assimilates to the damaged part to enable its repair.

The role of heat-induced action potentials in the control of CO_2 exchange in the leaves has been discussed later in this chapter along with KCl-induced signals.

4.4 Electrical Activity Induced in the Roots

The plant roots experience stress from time to time e.g. due to drought, water-logging etc. The results presented in this thesis clearly show that these conditions and many other factors do induce a depolarization of the transmembrane potential in the root cells which is a prerequisite for the elicitation of action potentials (Findlay and Hope, 1976).

4.4.1 Effect of KCl

KCl treatment of the roots, which was found to be the best method for inducing the action potentials, proved a very useful tool in understanding the nature and the mechanism of production of these signals.

The repetitive signals, induced in the roots due to KCl treatment and detected in the stem were quite variable in their amplitude (10-45 mV) and duration (15-45 s). However, their fast rise and slow fall to the base-line suggests that they are action potentials. Similar KCl-induced action potentials have been reported by Gunar and Sinyukhin (1962, 1963); Sinyukhin and Gorchakov (1966b); Kalinin et al. (1970); Opritov and Kalinin (1970); Pickard (1973); and Van Sambeek and Pickard (1976a). One important feature of the KCl-induced action potentials was that their total number and frequency depended to a large extent on the strength of the KCl solution. The results also show a threshold value of about 0.12 M KCl and a saturation value of about 0.8 M KCl. This threshold value and the intracellular potential measurements suggest that the cells need to depolarize to a critical extent before they can produce an action potential. Increase in the strength of KCl beyond the saturation point (0.8 M) led to a decline in the total number and frequency

of action potentials. This could have been due to the damage to or death of the excitable tissue. Passive diffusion of K^+ in the cells and tissue might be responsible for the fact that KCl treatment induces more signals than any other treatment. K^+ approaches an electrochemical potential equilibrium in a number of cases including higher plants and appears to be moving passively in accord with the electrical gradient (Higinbotham, 1973a). Kishimoto and Tazawa (1965) found that in *Nitella flexilis* the K^+ gradient across both the plasmalemma and tonoplast closely approaches the values predicted for a passive distribution. Although, there are some reports of active uptake of K^+ at lower concentrations (Etherton, 1963), generally, active transport is negligible at high external concentration in higher plant tissue (Higinbotham, 1973b; Pitman and Saddler, 1967). Increasing the K^+ concentration in the bathing medium has been shown to depolarize transmembrane potentials in *Chara* and maize root cells by Hope and Walker (1961) and Dunlop and Bowling (1971), respectively. The intracellular potential measurements, carried out in the present investigation, also showed an increasing depolarization of the transmembrane potential with increase in KCl concentration in the bathing medium. A sufficiently high depolarization could also induce an action potential. However, the whole of the depolarization should not be attributed to the passive diffusion of K^+ because an increase in osmotic pressure using mannitol was found to depolarize transmembrane potential of the root cells and it also induced a few action potentials in the roots of the whole plant. On the other hand, the osmotic effect of the KCl in the bathing medium might be opposed by the K^+ which diffuses into the cells passively. At this point it should be mentioned that it would not be right to make a close comparison between the effect of KCl and

mannitol on the transmembrane potential in order to evaluate depolarization caused by passive K^+ movement alone because there might be other factors involved in the process, e.g. passive diffusion of K^+ is significantly affected by the presence of Ca^{2+} in the medium (Spanswick et al., 1967; Higinbotham et al., 1964a, b) and at high external K^+ concentration there might even be an active efflux of K^+ (Higinbotham, 1973b). However, it can still be said that the depolarization induced by KCl may be due to both its osmotic effect as well as the passive diffusion of K^+ into the cells.

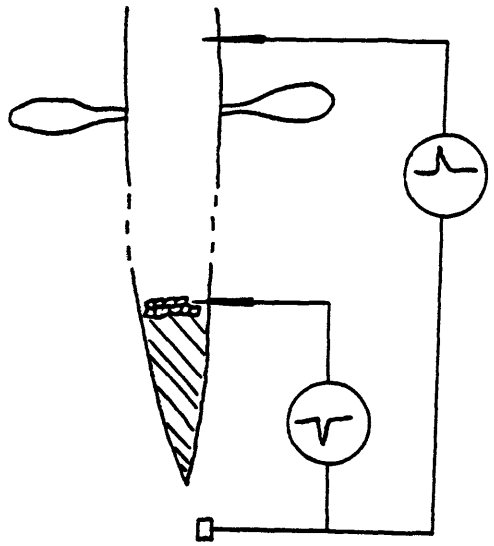
The fact that KCl treatment of the roots of the whole plant induces a large number of action potentials can be explained by assuming that K^+ diffuses into the root tissue both inward as well as upward. The rapid diffusion of K^+ into the tissue from cell to cell can be accomplished by the movement through the symplast and the apoplast (Aikman et al., 1980). As the K^+ moves into the tissue it depolarizes the cells progressively. When one of the cells has depolarized to its threshold level, it would produce an action potential and this might also trigger the neighbouring cells, which were nearing their threshold level, to fire with it in near synchrony. This is likely to happen due to the electrotonic transmission of the signal from cell to cell via the plasmodesmata. The positive-going potential within the firing cell will be transmitted by instantaneous ionic conduction via the plasmodesmata to the neighbouring cell and this may be sufficient to trigger it to fire synchronously if it is already near its critical level. Further movement of the "K^+-front" would depolarize more cells and other action potentials will be fired. However, it cannot be said whether or not a depolarized cell would fire an action potential more than once. It is possible that the "K^+-front" finally reaches the

junction of root and stem and moves further into the stem inducing new action potentials there too. Evidence that the stem tissue can also be stimulated to produce action potentials with KCl comes from the work with isolated stem-sections which were also found to be capable of generating action potentials when stimulated by KCl. Thus, it seems that excitable cells in any tissue when depolarized beyond a threshold level can produce action potentials. A phenomenon, similar to the KCl-induced action potentials in higher plants, also occurs in the cells of *Acetabularia*. Gradmann and Mummert (1980) reported that the frequency of the spontaneous action potentials in *Acetabularia* can be increased by increasing the K^+ concentration in the bathing medium. Thus, the suggestion is that most types of cells can be induced to produce action potentials by KCl treatment.

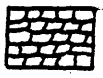
The KCl-induced action potentials do not seem to show the true propagation characteristics of heat-induced action potentials. Their conduction in the plant must be electrotonic since the action potentials were always of positive polarity and were always detected in absolute synchrony at widely distant points on the stem. However, during electrotonic conduction in the shoot, the degeneration of the action potential was very slight and was barely noticeable even when the electrodes were placed as far as 15 cm apart (Figure 3.26). This might be due to the fact that the action potentials are large, that a great number of cells are involved by firing in synchrony and that the electrical path in the shoot offers little resistance to electrotonic conduction. The action potentials could be detected at almost any part of the shoot including leaf blade, tendril, stem and petiole. That the conduction in the shoot is electrotonic is further supported by the fact that the recording electrode placed on the

leaf blade away from any major veins recorded the action potentials simultaneously with another electrode placed in the stem. The only part the vascular bundles seem to play in the electrotonic conduction is that they provide channels of somewhat lower resistance than the rest of the tissue, which was indicated by the slightly larger size of the action potentials inside the vascular bundles than outside. This might be due to the fact that the vascular bundles contain sieve tubes with their large sieve pore connections. Thus, the observations in the present investigation, on the non-propagating nature of KCl-induced action potentials, are in disagreement with the reports by Gunar and Sinyukhin (1962, 1963) and Sinyukhin and Gorchakov (1966b). The discrepancy on the nature of these action potentials may be due to the incorrect interpretation, by the Russian workers, of the artifacts which might have arisen due to the misplaced reference electrodes. This has also been pointed out by Van Sambeek and Pickard (1976a), who observed KCl-induced action potentials but found no evidence of their propagation.

The application of KCl to the roots of a plant induced action potentials in them which were of positive polarity as detected in the shoot by an Ag/AgCl or a tungsten electrode, and negative polarity as detected in the root by a tungsten electrode (Figure 3.29). The two kinds of polarity are explicable because the transmission of this type of action potentials over a distance is electrotonic. Since the electrode placed in the stem was very far away from the root cells firing the action potentials, the action potential recorded on it would be of positive polarity due to passive electrotonic transmission in the shoot as explained in section 4.2.1. However, the apoplast near the firing cells becomes negative (perhaps due to leakage of negative ions) during an action potential,



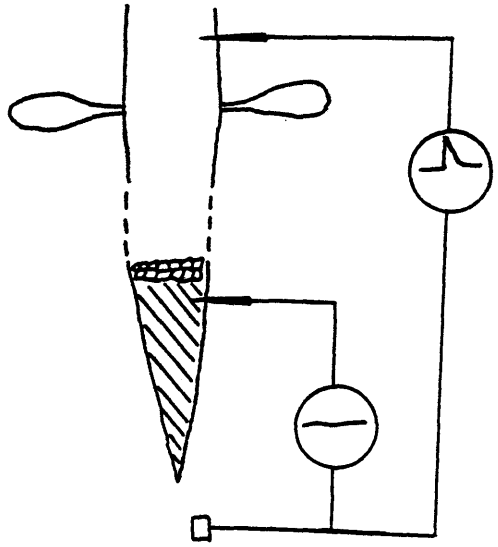
A



"K^+-front" or the cells firing action potentials



Tissue penetrated by KCl



B

Fig. 4.1

and therefore an electrode placed in the root in contact with the apoplast would also register the action potential in its negative polarity (Figure 4.1a). Further evidence that the firing cells were very close to the recording electrode in the root comes from the fact that this electrode only registered the first action potential, and the rest were detected by the electrode in the stem. This could have happened if a short circuit developed between the recording electrode in the root (for this particular experiment, most of the root system was submerged in the bathing medium except for the upper part of the root, in which the recording electrode was implanted) and the reference electrode due to KCl-penetration (Figure 4.1b). However, the electrode in the stem would still register the action potentials which were being produced by the more proximal part of the root and finally the lower end of the stem.

Although, the intracellular potential measurements were made only on the cortical cells in the root, it would not be wrong to assume that most cells with large transmembrane potentials in the root and shoot can be stimulated by KCl to produce action potentials.

4.4.2 Effect of Osmotica

The results show that in the first 20 minutes NaCl was as effective as KCl, in inducing action potentials in the roots of a plant, but in contrast to the KCl-induced signals they soon died out. This could be explained by assuming that the initial response to the NaCl treatment was due to its osmotic effect and perhaps some passive diffusion of Na^+ into the cells as described for the KCl-treatment. However, the evidence in the literature suggests a passive influx to be less likely. Unlike K^+, Na^+ fails to accumulate in the cytoplasm of either plant or animal cells, along the

electrochemical potential gradient and there is clear evidence from electrochemical measurements that the diffusive permeability of the plasmalemma to Na^+ is much lower than to K^+ (Pitman, 1976). In addition, an efflux pump for Na^+ at the plasmalemma has been suggested (Findlay et al., 1969; Higinbotham et al., 1967; Pierce and Higinbothan, 1970). If, consequently, the main effect was osmotic, maybe due to plasmolysis, it might explain to why unlike KCl, further increase in the NaCl concentration did not increase the number of action potentials. However, it is possible that there might be other reasons for NaCl not being as effective as KCl.

$CaCl_2$ was less effective than either KCl or NaCl in inducing action potentials in the roots of a plant. $CaCl_2$ induced only one action potential even at a concentration as high as 0.5 M. Probably the only effect $CaCl_2$ treatment had in inducing action potentials was osmotic because there would not be an appreciable passive diffusion of Ca^{2+} into the cells as the permeability of the plasmalemma to this ion is very low (Spanswick and Williams, 1965). Furthermore, an increase in Ca^{2+} in the bathing medium has been shown to induce a hyperpolarization in oat coleoptile cells (Higinbotham et al., 1964a) and would be expected to actually inhibit action potentials. Thus, the results of experiments with NaCl and $CaCl_2$ treatment also indicate, although indirectly, that the large number of action potentials induced by KCl treatment was mainly due to the K^+ ion and its ability to rapidly diffuse into the cells.

It would be apposite here to briefly elucidate the events which follow the increase in osmotic pressure around a walled cell. When the external osmotic pressure is suddenly increased around a walled cell, the fluid flows out, the volume decreases, and the wall shrinks until the decreased pressure it generates equals the reduced

osmotic pressure difference between the inside and outside the cell. At this stage the cell is in equilibrium with its environment. It has been found that some cells treated in this way subsequently recover their initial turgor. This is achieved by their increasing their internal osmotic pressure. Osmotica are accumulated by transport or/and biochemical interconversions. In many higher plants the main osmoticum is again K^+, most often associated with organic acids (Cram, 1976).

Mannitol treatment of the roots of a plant induced a few action potentials. These action potentials may have been induced as a result of the depolarization caused by the increase in osmotic pressure around the root cells. Goring et al. (1979) have shown a depolarization of the transmembrane potential with increase in external osmotic pressure in the cells of wheat and corn coleoptiles. Indeed, the results of intracellular potential measurements, in the present investigation, showed a depolarization of the transmembrane potentials of root cells with increase in osmotic pressure in the bathing medium and a high enough depolarization also elicited action potentials. Since mannitol treatment of the roots of a whole plant induced only a few action potentials, it seems likely that the membrane potential begins to repolarize once the osmotic equilibrium is reached and no more action potentials are induced. A combination of mannitol and KCl had a stimulatory effect on the number and frequency of action potentials which suggests that two different factors which induce depolarization by different mechanisms can have an additive effect when applied together.

The osmotic shock-induced depolarization might have been due to its effect on the events regulated by the plasmamembrane. Rubinstein et al. (1977) observed a small reduction in the uptake of

Cl^- as a result of osmotic shock. Since the rate of respiration was not affected by the osmotic shock, it is unlikely that the reduced Cl^- uptake was accounted for by reduced ATP availability. The Cl^- pump must be affected by some other mechanism, and it is conceivable that permeability changes towards other ions could also be affected in a similar way.

It appears that the membrane potential, along with the turgor pressure, probably recovers, to some extent if not fully, after a period of one hour or more. Evidence of this is that the removal of the osmoticum, by replacing the mannitol with normal bathing medium or watering a wilted plant, also induced action potentials. This could only happen if the membrane potentials of the root cells was depolarized again beyond the threshold level and implies that drastic turgor change in either direction elicits the response. A similar observation was reported by Zimmermann and Beckers (1978) in *Chara corallina*. Using a pressure probe they showed that a momentary increase or decrease of pressure gradient inside the cell, both led to a depolarization of the membrane potential and when the gradient exceeded 2 bar, action potentials were initiated. Thus, at the moment it can be said that application or removal of osmotic pressure could have an effect on either electrogenic pumps or passive ion transport pathways across the membrane, which leads to depolarization. It is also possible that the osmotic shock-induced action potentials are a direct result of slight membrane damage which might occur during turgor changes, and they are produced in a way analagous to the injury-induced action potentials. However, it remains to be seen whether the mechanism of depolarization (e.g. types of ions involved) in both cases is the same or different.

Such a depolarization and the resultant action potentials would have a significance in communication with the other parts of the plant, if the action potentials were of a propagating nature. However, the results in this thesis suggest that the action potentials induced by means of osmotic stress as well as saline treatment do not propagate in the plant and are only detected because of electrotonic conduction. Mummert and Gradmann (1976) speculated, on the basis of simultaneous flux and potential measurements in *Acetabularia*, that action potentials which occur spontaneously in this species have a significant function for the K^+ balance and, in turn, for turgor pressure regulation. It is possible that the depolarization and/or the non-propagating action potentials induced in the roots by pressure change and salinity might have a similar local function, perhaps in triggering some biochemical and biophysical regulatory processes which contribute to osmoregulation. However, since an increase or a decrease in osmotic pressure both cause depolarization and action potentials, it would be difficult to say at the moment how they can bring about the different regulatory changes which would be needed to meet two opposite situations.

Attempts to detect action potentials from wilted plants in dry soil did not succeed. This could have been due to the poor contact of the reference electrode with the roots of the plant because of the dryness of the soil. It is also possible that the depolarization and hence the action potentials are induced only as a result of sudden osmotic shock and not due to a gradual slow change in the osmotic pressure which happens during the drying of the soil.

4.4.3 Effect of Growth Substances

4.4.3.1 Auxins

IAA or 2,4-D (10^{-4} M) treatment of the roots of a whole plant did not induce any action potentials. Intracellular potential measurements showed that IAA at concentrations of 10^{-6} and 10^{-5} M induced a small (about 20 mV) depolarization of the membrane potential in a few minutes. Clearly, the depolarization induced by IAA is too small to induce any action potentials. Goring et al. (1979) have reported a similar IAA-induced depolarization of cell membrane potential in wheat and corn coleoptile cells. However, these observations are in disagreement with the reports of Etherton (1970) and Cleland et al. (1977) who showed a hyperpolarization of the membrane potential as a result of IAA application. The discrepancy in the results can be resolved if the time, when the potential difference was measured after the IAA application, is taken into account. The hyperpolarization reported by Etherton (1970) was based on membrane potential measurements made 2-8 hours after the application of IAA. Even Cleland et al. (1977), who reported an IAA-induced hyperpolarization of the membrane potential of about 25 mV in oat coleoptile cells, observed a small initial depolarization of about 5 mV. Thus, it may be said that the initial response to IAA-application is a small depolarization which under some conditions might be followed by a hyperpolarization. This conclusion is supported by the report of Morath and Hertel (1978) on the development of lateral electrical potentials following asymmetric auxin application to a maize coleoptile. They observed an early negative potential change of about -1 mV at the surface on the side of a strong auxin application. This negative potential lasted for about 15

minutes and was followed by a strong and long-term positive lateral potential of up to +12 mV at the auxin-treated side. The initial auxin effect appeared to depend on the size of the step-up in auxin concentration. These results could be explained if IAA induced a small depolarization first followed by a large, long-lasting hyperpolarization. It is possible that the IAA-induced depolarization somehow activates long-term H^+-efflux which then leads to a hyperpolarization. Hager et al. (1971) proposed that auxin is an effector of a "proton-pump" which transports or secretes protons into the cell wall compartment and requires respiratory energy (ATP) for its operation. The accumulated protons in the cell wall would then trigger off an enzymatic process, causing a loosening of the cell wall material which finally leads to cell elongation. These findings have been confirmed by Cleland (1973, 1975), who demonstrated that within 15 to 20 minutes of its application, IAA could induce excretion of H^+ from coleoptile cells.

The roots of a plant pretreated with IAA could be induced by KCl to produce a larger number of action potentials than without the pretreatment. IAA was just as effective at 10^{-12} M concentration as it was at 10^{-6} M. Another dramatic effect of the IAA treatment was that the latent period, between the application of KCl and the arrival of the first action potential, was reduced to a few seconds. This suggested an increased permeability of the cells to K^+ after IAA pretreatment. Indeed, it was confirmed by intracellular potential measurements which showed that the depolarization induced by KCl treatment after the IAA pretreatment was much bigger. This conclusion that IAA increases the membrane permeability to K^+ is further supported by the report of Van Steveninck (1974), who demonstrated an IAA-effect on ion selectivity favouring K^+ uptake over

Na^+ uptake which was opposite to the effect of ABA. Similar results were also obtained by Cocucci et al. (1976) with the phytotoxin, fusicoccin(FC) which elicits many IAA-like responses, e.g. hyperpolarization of the transmembrane potential, proton secretion, growth promotion etc. They found that the K^+-induced depolarization is greater in the presence of FC and suggested that FC increases the selectivity of the transport system for K^+. Whether or not the IAA-induced K^+-influx is coupled to the H^+-efflux is, however, a controversial issue. Hager et al. (1971) suggested that the transport of protons into the cell wall should be compensated by a flow of other cations towards the interior of the cell (e.g. K^+ for H^+ exchange) or by a flow of anions to the cell periphery in order to maintain electrical neutrality. In that case K^+-influx would be a secondary effect of the hyperpolarization. On the other hand, Ilan (1973) observed an auxin-induced pH drop of the external solution within 2-3 hours if the sunflower hypocotyl segments were floated in a K^+-containing solution. Because of the apparent secondary nature of this H^+-efflux, Ilan (1973) believed that IAA-stimulation primarily concerned the influx of K^+. It is possible that K^+-requirement which Ilan (1973) observed was for the initial IAA-induced depolarization of the cells which would then activate the H^+-efflux leading to hyperpolarization. The H^+-efflux again may be coupled to K^+-influx. However, the idea that K^+ is needed for the initial depolarization is only speculative and whether or not this happens must await further work.

In conclusion it may be said that IAA at physiological concentrations has some direct effect on the transmembrane potential but a profound effect on the permeability of the membranes to ions like K^+.

4.4.3.2 Abscisic Acid (ABA)

There is some divergence in the published experimental results on the effect of ABA on ion transport. Van Steveninck (1972) showed that ABA promotes the net uptake of K^+, Na^+ and Cl^- in aged beet root slices and also changes the K^+-Na^+ selectivity to favour Na^+ uptake. On the other hand, ABA seems to control stomatal closure by promoting the movement of K^+ out of the guard cells (Jones and Mansfield, 1970; Mansfield and Jones, 1971). In certain circumstances ABA leads to the inhibition of transport of ions through the root to the xylem in maize and barley (Cram and Pitman, 1972), where it does so by acting primarily on the release of ions into the xylem (Pitman et al., 1974). In the present investigation ABA treatment of the roots of a plant did not induce any action potentials, nor did ABA pretreatment have any effect on the number of action potentials induced by KCl. This suggests that it has little or no short-term effect on the movement of K^+ into the root tissue. In conclusion it may be said that ABA has specific effects on ion transport at specific sites in the plant but the present investigation did not provide evidence for any short-term effect in *cucurbita* roots.

4.4.3.3 Gibberellins and Cytokinins

GA or kinetin treatment of the roots of a *cucurbita* plant did not induce any action potentials, nor did the pretreatment with these have any obvious effect on the number of action potentials induced by KCl. Therefore, it seems that the membrane permeability changes, which these hormones are supposed to induce, either do not affect the membrane potentials or they are not short-term effects as in the case with IAA. The reported changes in permeability were

the long-term effects of these hormones and possibly involved prior changes in the DNA-RNA-protein system and probably growth (Ilan et al., 1971; Kohler, 1970).

4.4.3.4 Acetylcholine (ACh)

Acetylcholine, which functions as one of the neurohumors in animal systems, induces ion fluxes and sometimes action potentials in them. ACh is widely distributed in plants too, and occurs in almost every organ (Jaffe, 1970; Hartmann and Kilbinger, 1974). ACh also elicits IAA-like responses in plants, e.g. induction of H^+-efflux (Jaffe, 1970), and stimulation of elongation of *Avena* coleoptiles (Evans, 1972). In the present investigation, ACh application to the roots of a plant did not induce any action potentials. It is possible that ACh does not cause the necessary depolarization which is prerequisite for the production of action potentials. If it works by directly promoting active H^+-efflux, it is more likely to cause a hyperpolarization. However, there was some increase in the number of KCl-induced action potentials in the roots of a plant pretreated with ACh. Two reasons could account for this. Either ACh caused a small depolarization which was additive with the KCl-effect or it increased the permeability of the cells to K^+. The first possibility seems less likely as Hartmann (1977) found no short-time effect of ACh on the extracellular bioelectric potential differences of etiolated *Phaseolus vulgaris* hypocotyls. The possibility that ACh increased passive permeability to K^+ and that K^+ uptake is coupled to the ACh-induced H^+-efflux seems more likely. Jaffe (1970) observed that the efflux of ACh and its endogenous level was increased in the roots of *Phaseolus aureus* by red light-exposure, and ACh mimicked many of the red light-responses, including

H^+-efflux and adhesion of the root tips to a negatively charged glass surface. ACh may require metabolic energy to function as Yunghans and Jaffe (1972) showed that ACh increases O_2 uptake and causes rapid utilization of ATP pools. Jaffe (1970) concluded that phytochrome-responses are mediated by ACh and that ACh functions in mung bean roots as it does in animal systems, i.e. by mediating changes in ion flux across the cell membranes by regulating their permeability. However, some intracellular potential measurements are required to further understand the mechanism of ACh-action.

4.4.3.5 Ascorbic Acid (AA)

Ascorbic acid which is a reducing agent occurs naturally in most plants and animals and serves important functions in many systems. AA treatment (100 mM) was found to induce leaf closure in *Mimosa* (Hesse et al., 1957). In the present investigation AA induced action potentials in the roots of a plant but only at a very high concentration (100 mM). This effect of AA was not due to its acidity. Intracellular potential measurements further showed that AA induced a depolarization of the membrane potential. Ascorbic acid is involved in so many systems in plants that it would, as yet, be difficult to pinpoint the exact mechanism by which it induces the depolarization of the membrane potential which is required for the production of action potentials. One explanation is that AA affects the membranes directly by reducing the disulphide bonds in the protein component to sulphydryl groups. This type of change in the protein component may alter the membrane permeability. On the other hand, AA might affect the membrane permeability indirectly also. The ascorbate anion has a tendency to form a complex with cations like Ca^{2+}, Mg^{2+} as well as other metallic ions (Lewin, 1976).

Removal of Ca^{2+} in the form of complexes could allow a greater diffusion of K^+ into the cells, thus depolarizing them. It is also possible that AA-induced depolarization involves some redox reactions at the plasmalemma. Mitchell (1961) suggested that the energy of biological oxidation-reduction reactions can appear directly as a transmembrane potential difference and the membranes of chloroplasts and mitochondria are already known to contain such well-developed redox reaction systems.

The combination of AA with KCl induced more action potentials in the roots of a plant than these factors would individually, thus indicating once again that the depolarization induced by two different factors may have an additive effect on the number of action potentials. The lack of K^+ in the bathing medium seriously affected the ability of the roots of a plant to produce AA-induced action potentials. This observation is supported by the report of Vyskrebentseva and Sinyukhin (1967), who demonstrated that evocation of action potentials is difficult or impossible in potassium-deficient pumpkin plants. However, at this stage it cannot be said whether the lack of K^+ in the bathing medium affects the initial depolarization process or the actual production of action potentials.

Although the concentration of AA required for the production of action potentials is very high (unphysiological), nevertheless, the use of AA in inducing action potentials may serve a very useful tool in understanding the mechanism of the production of action potentials in higher plants, especially if it is used with intracellular potential measurements.

4.4.4 Effect of Anaerobiosis

Anaerobic treatment of the roots of a plant lasting up to

40 minutes did not induce any action potentials. However, the number and frequency of action potentials induced in the roots by KCl during the anaerobic treatment was quite high compared to KCl treatment alone, thus suggesting that the anaerobic treatment on its own might be depolarizing the membrane potentials of the cells but not to the threshold level. This prediction was confirmed by the results obtained from the intracellular potential measurements. Anaerobiosis reversibly depolarized the membrane potential by about 40 mV, which, it seems, is not enough to initiate action potentials. The anoxia-induced depolarization reported for the cells of shoots are much higher than those of the root. Okamoto (1976) observed a depolarization of about 60 mV in the leaf cells of *Antirrhinum* under the effect of anoxia. A large anoxia-induced depolarization of about 70 mV was detected in the parenchymatal cells of *Vigna sesquipedalis* hypocotyls and on rare occasions the depolarization led to action potentials (Katou, 1978). It is possible that the roots are more likely to be resistant to the effect of anoxia, e.g. by utilizing energy from anaerobic respiration, because they often encounter such a situation naturally in the soil. Support for this view comes from the reports of Vartapetian et al. (1978), who demonstrated anaerobic respiration in mesophyte roots. However, in the long-term anaerobiosis has a depressing effect on the normal functioning and the growth of the roots as well as the whole plant. Vartapetian et al. suggested that the depressing action of long-term anaerobiosis on the mesophyte roots is not due only to inhibition of the aerobic utilization of respiratory substrate, but also to a more rapid exhaustion of the stores of organic substances brought about by anaerobic respiration, which is not compensated by an influx of assimilates from the leaves. Such an adaptation to be

able to fall back on anaerobic respiration must be of great significance to the roots which often face anaerobic conditions.

A surprising result was obtained, in the present investigation, when the respiratory inhibitor cyanide was used. KCN (10 mM) treatment of the root cells led to a much bigger depolarization of the membrane potential than the anaerobic treatment. A similar observation has been made in *Neurospora crassa*, where the membrane potential depolarized from -200 to -100 mV in response to anoxia, but almost to zero in response to cyanide (Slayman, 1965). Although oxygen tension was not measured, Slayman postulated that the incomplete depolarization was because his experimental conditions may not have totally excluded oxygen, and a similar explanation might be proposed for the result of the present work. However, this explanation is not altogether satisfactory since both tissues are capable of short-term anaerobic respiration (Vartapetian et al., 1978; Cochrane, 1958; Blumenthal, 1965) which should have maintained some sort of membrane potential even in the presence of cyanide. It is therefore conceivable that this inhibitory effect of cyanide might be due to some mechanism other than simply a reduction in the ATP supply. Support for this view comes from the report of Higinbotham and Anderson (1974) who suggested that the inhibitors like cyanide and azide clog the entire electron transport chain and in addition to inhibiting ATP synthesis, they also affect ATPase activity. Since the ATPases are present at the membrane transport site and if they are involved in active transport, an inhibition of their activity should lead to a larger depolarization than when just the ATP supply is reduced. However, further information, regarding the ATP level under the two conditions is needed to confirm the assumptions made above.

4.4.5 Effect of cyanide

The inhibition of respiration by potassium cyanide (KCN) treatment of the roots induced signals which could be detected by electrodes placed in the stem, suggesting that KCN induces a depolarization of membrane potential which is large enough to initiate action potentials. Jones et al. (1975) have observed a similar phenomenon. They reported that depolarization and action potentials were produced as a result of cyanide treatment of both nematode-induced transfer cells and normal cells of cotton cotyledons. The intracellular potential measurements, in the present investigation, showed that KCN (10 mM) induced a large depolarization as well as some action potentials, thus lending support to the findings of Jones et al. (1975). The residual potential, after the total inhibition of respiration by KCN, was probably a passive diffusion potential. However, this residual potential was quite small (about 15 mV), suggesting that a large portion of the cell membrane potential is electrogenic in origin and depends on respiratory energy (Higinbotham et al., 1970).

Thus, the results of the present investigation show that many factors can cause the depolarization of the membrane potential which is necessary to initiate action potentials. Two or more factors can have an additive effect on depolarization and, in turn, on the action potentials. Because of their non-propagating nature and the fact that depolarization caused by any means (including cyanide) can induce them, it seems that the action potentials described in section 4.4 represent the primitive form and it is possible that from these the propagating type (injury-induced) of action potentials arose. However, since plants show both types of action

potentials they might have different functions.

4.5 KCl and Heat-induced Action Potentials and the Rate of CO_2 Exchange

As discussed before, the KCl-treatment of the roots of a plant induced action potentials in them which did not propagate in the stem but were only detected because of electrotonic conduction. Although, the KCl-induced action potentials in the roots were detected in the petiole of the leaf being used for CO_2 exchange measurements, the leaf showed little or no change in the rate of respiration and photosynthesis. The small, slow and steady decline in the rate of respiration could have been due to the damage to the plant by KCl treatment. The non-propagating nature of these action potentials, itself argues against their being a means of communication with the other parts of the plant. If these action potentials in the roots do have a function, it might be a local function, perhaps in osmoregulation as has been suggested before.

The heat stimulus to a leaf induced a series of large action potentials which could propagate down the petiole and spread into the stem and the neighbouring leaves. However, these propagating action potentials, produced by heat damage to a leaf, did not have any effect on the rate of respiration of a neighbouring leaf even though they could be detected in its petiole. It is possible that the increase in the rate of respiration, which other workers have observed, is in response to the variation potential and not the action potentials and since the variation potential was not observed, in the present investigation, no change in the rate of respiration was observed. Confirmation for this interpretation comes from the reports of Van Sambeek and Pickard (1976c) who found that the change

in the CO_2 exchange coincided with the arrival of the variation potential and not the occurrence of action potentials. Thus, it seems that the heat-induced variation potential if produced and transmitted to the neighbouring leaves may lead to the changes in CO_2 exchange but the action potentials which are always produced in response to heat or some other form of injury have no control over the rate of CO_2 exchange of the neighbouring undamaged leaves.

The evidence presented in this thesis clearly shows that the electrical activity in plants manifests itself in various forms, e.g. steady potentials and action potentials. Steady potentials are of different kinds and can be induced in response to different factors, e.g. phytochrome-mediated potentials and IAA-induced potentials. There are different types of action potentials too depending upon their size, duration, nature of propagation and mechanism of production. Although, no clear function for the action potentials has emerged from the present investigation, it can be said that there are many natural factors which can induce the action potentials either individually or in combination and depending on their nature the action potentials might have different functions.

The evidence presented and the various other reports discussed in this thesis have broadened our understanding of the nature of the different types of electrical activities, and this should serve as a good background for the future research.

Summary

1. Red light induced a rapid positive change in potential at the tip of wheat coleoptile. FR had a reverse effect. FR was ineffective without previous R.
2. R-FR-induced potential changes take place entirely between the tip and the base of the coleoptile, and roots are not involved. All the parts of the coleoptile are equally perceptive.
3. Decapitated coleoptile lost the ability to respond electrically to R-FR. IAA substituted in place of the tip did not maintain the response.
4. The shoot of light-grown marrow plants showed 4 types of electrical activity, viz. trains of repetitive spiking, "irregular" signals, complex activity, and the rare "solitary" signals. Trains of repetitive spiking were found mostly in the young growing parts of the shoot.
5. The electrical activity observed in the shoot does not seem to be related to the phloem activity.
6. Auxin, ACh and ABA do not seem to affect the spontaneous electrical activity in the shoot in short-term.
7. A heat stimulus to a leaf or the roots induced one or more propagating action potentials.
8. KCl-treatment of the roots of a whole plant, or isolated stem sections induced a number of large, non-propagating action potentials which could be detected due to electrotonic conduction. The number and the frequency of the action potentials seem to depend on the strength of KCl used. The effect of KCl seems to be partly osmotic, the major effect being due to passive

diffusion of K^+ into the cells.

9. A sudden increase or decrease in osmotic pressure around the root cells, both induced action potentials.
10. An anaerobic environment around the roots induced a depolarization of 40 mV, which, it seems, is not large enough to initiate action potentials.
11. IAA (10^{-5} M) induced a small depolarization in a few minutes in the root cells. IAA, at even as low a concentration as 10^{-12} M, seems to increase the membrane permeability to K^+.
12. GA, kinetin and ABA seem to have no short-term effect on the membrane permeability to K^+.
13. Ascorbic acid induced a depolarization of the cell membrane potential in the root and at high concentration induced action potentials.
14. KCN induced a large drop in the membrane potential and produced action potentials in the roots.
15. KCl-induced action potentials in the roots did not have any short-term effect on the rate of photosynthesis and respiration in leaves.
16. Heat-induced propagating action potentials did not affect the rate of respiration of undamaged neighbouring leaves.

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