J. Rubb. Res. Inst. Malaya, **21(4)**, 524–542 (1969)

Electrophysiological Phenomena in Hevea Brasiliensis

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Electrical potentials can be detected in Hevea brasiliensis *as a response to wounding. The response can be detected only very near the wound and there is no mechanism for distant transmission as observed in* Mimosa pudica. *A comparison between young* Hevea *seedlings, mature* Hevea *trees and* Mimosa pudica *shows that the electrical response patterns to mechanical wounding are otherwise broadly similar. Experiments on* Hevea *following various treatments show that the response is elicited only from living tissues, and that it can be temporarily inhibited by certain chemicals, especially chloroform and ether. Possible implications of the phenomenon are discussed in relation to latex flow.*

This work was undertaken as part of a programme of research on the mechanism by which latex flow is terminated after tapping. Arising from these studies a hypothesis was advanced that depolarisation of active polarised membranes within the latex vessel might act as a trigger for release of materials across membranes, starting a chain of events leading to blockage of the vessel. There are indications that whatever mechanisms may be responsible for vessel plugging, they are strongly localised near the tapping cut (BOATMAN, 1966). On this basis, a wound response from *Hevea* detectable as a change in electrical potential and localised near the wound was predicted. Initially, it was intended to test this prediction and it was in fact verified. The existence of electrical effects of this nature is of considerable interest and has not been studied previously in *Hevea*. These studies were undertaken to establish the main outlines of the phenomenon as exhibited in *Hevea*, to relate it as far as possible to known electrical responses in other plants and to ascertain whether the response could be explained in terms of purely physical agencies (such as concentration gradients or streaming potentials) or whether it involved membrane activity. In the latter case, it could be expected that chemicals known to affect electrical phenomena associated with membranes in other tissues would temporarily inhibit such responses, and that death of the tissues would remove them completely.

* *née* Weston

EXPERIMENTAL

An electrical potential cannot be measured except in relation to some reference potential. For work on plants the alternatives are to have the reference electrode at the plant itself or at earth potential in the soil. In both cases the situation is complicated by the fact that the stems of the higher plants show appreciable electrical potentials with respect to earth ('stem potential'). Stem potentials for a given plant show variations according to the location on the stem where the measurement is made; also, for a given location, the potential varies with time (though usually not very quickly). All higher plants which have been investigated show these stem potentials; some have been studied in detail (BOSE, 1927; CLARK, 1938; FENSOM, 1963; LUND, 1931a and b; ROSENE, 1937; REHM, 1938; SCOTT, 1967; ETHERTON AND HIGINBOTHAM, 1960).

Thus, where wound potentials exist in a plant, they must be detected as relatively weak transient signals superimposed on a slowly fluctuating base signal.

A variety of electrode systems has been used for plant studies, the simplest being a metal probe, usually of silver/silver chloride. Robust

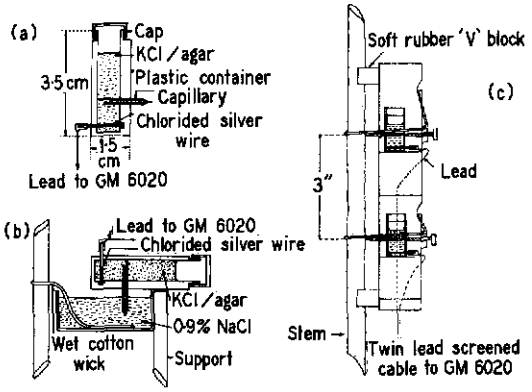


Figure 1. (a) shows internal electrode, (b) external electrode, (c) lightweight polystyrene foam platform clad with 1/32" gauge aluminium used as holder for two internal electrodes and attached to stem of plant.

chlorided 'silver nail' electrodes are easy to use, but for work in a new field, they could be open to criticism as possibly giving rise to junction potential artefacts. The work was hence carried out with silver/silver chloride electrodes connected to the plant through salt/agar bridges, the terminal connection to the plant being either salt/agar or salt solution. The salt/agar gel used was 0.7% agar-agar in 3 M KCl (223.5 g KCl, 7 g agar-agar, 5 ml glycerol and 1 g phenol per litre). The silver wires were chlorided electrolytically for 15 minutes in concentrated HCl. The authors used these in two forms — one ('internal electrode') was intended to pick up signals from the tissues of the plant ('internal potential') and the other ('external electrode') was designed to pick up any signals which could be detected at the outer surface of the plant ('external potential'). The use of an internal electrode involves wounding the plant to insert it. The external electrode avoids the need for such wounding. The two systems are shown in *Figure 1 (a* and *b)*. For work on fully grown *Hevea* trees, a slightly different type of internal electrode was sometimes used to cope with the high turgor pressure (10–15 atmospheres) (BUTTERY AND BOATMAN, 1964) which tended to force latex through the capillary connection, displacing the agar gel before it. This is shown in *Figure 2*, the latex being allowed to flow freely over the top of the agar gel so that the final electrical connection to the tissues was through the latex itself.

The capillary electrode tips were produced by drawing out haematocrit capillaries in a simple laboratory-made micro-forge. Experiments were made initially with very fine tips but the results did not justify the effort and tips of about 250 μ bore upwards were found more practicable. The internal electrodes were checked in pairs before use by immersing their tips in a 1 M solution of KCl. Electrodes were rejected if this test showed a potential between any pair in excess of 5 mV. Long-term viability of the internal electrodes was rather difficult to attain in the type of experiment carried out; the effective life was usually limited to a few hours and occasionally to a few minutes. However, in a few instances long-term recording was possible. The end of electrode viability was characterised by higher ratic potentials, often of opposite polarity to the reading given immediately before breakdown,

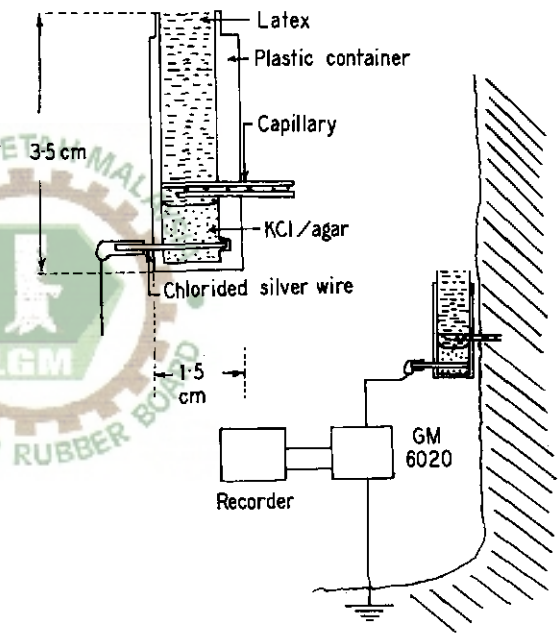


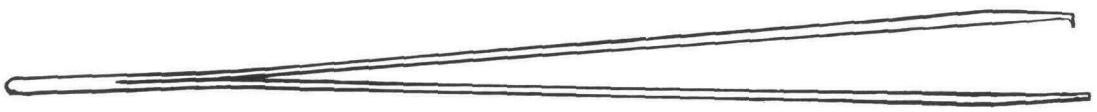
Figure 2. Electrode used for mature Hevea *trees, the capillary is not drawn out and enters the cell above the KCl/Agar, latex flooding the surface of this and making the terminal contact.*

and by a sudden failure of the recorded potentials round the plant/soil/electrode circuit to balance even approximately in accordance with Kirchhoff's laws. This meant temporarily abandoning the particular experiment, and investigation as to the cause of breakdown usually revealed air bubbles in the capillary or physical blockage of the tip. Fortunately, the symptoms were easily recognised.

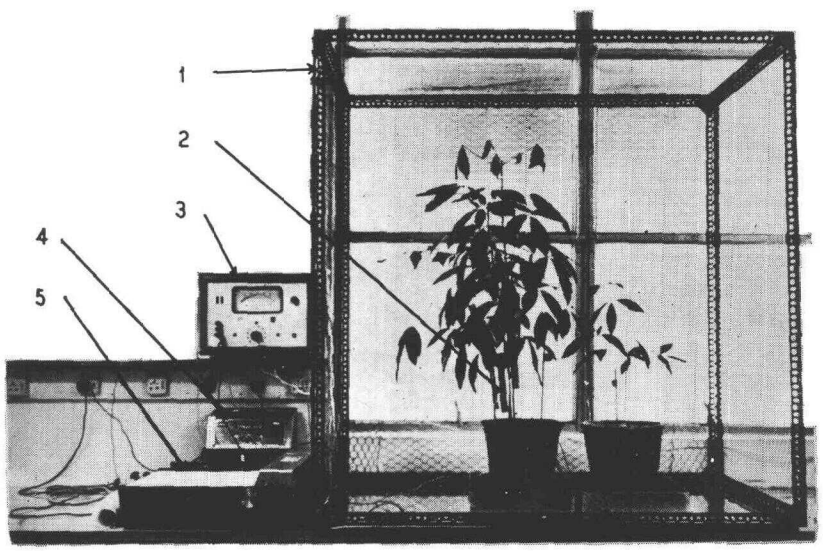
For work on the wounding of small plants such as *Mimosa pudica* or *Hevea brasiliensis* seedlings in pots, a device was constructed from a pair of tweezers to give a small standardised wound. The body of the tweezers was insulated with PVC tape. One arm was slightly shortened, ground to a needle point, and bent at right angles to give a short spike (1 mm long), while the other arm was left intact to give support at the back of the stem. By gripping the stem gently in this device, a more or less standard wound was inflicted without shaking or bending the stem in the process *[Figure 3(a)]*.

Connections from the electrodes to the recording system were made through lightweight screened cables supported near the plant. For laboratory work with pot plants, the plants were brought into the laboratory just before the experiment and the actual work was carried out in a grounded wire-mesh screening cage to reduce disturbances from stray fields in the laboratory *[Figure 3(b)]*.

For work on pot plants of *Hevea* whose



(a) Wounding tweezers



(b)

Figure 3. (a) shows tweezers modified to give a standard wound when a small stem is gripped between the prongs. The tweezers were insulated with PVC tape except for the tips to reduce disturbances from the operator. (b) shows the general arrangement for work with young plants (1—screening cage; 2—electrodes; 3—meter/amplifier; 4—voltage stabiliser; and 5—pen recorder).

stems were capable of taking the weight, the internal electrodes were supported in pairs from the plant stem itself in a light-weight screened holder which included screw devices for driving the capillary into the plant *[Figure 1(c)]*. This method had an advantage in that the electrodes moved with the stem if the plant swayed or was knocked. Relative motion between an internal electrode and the stem would result in a new electrode wound. For *Mimosa*, this technique was not practicable and the electrodes had to be supported independently on micro-manipulator heads near the plant, which was tethered to prevent relative movement. For *Mimosa* most of the work was therefore done with external electrodes which presented no problems because no electrode wounding arose from their use.

The basic electrical circuit is shown in *Figure 4* for one electrode at the plant and one in the soil. The signal was taken to a Philips GM 6020 microvoltmeter. This instrument has two input impedances and a number of ranges; the input impedance used was 100 m Ω. Direct readings could be taken from the meter scale, or alternatively the instrument output (meter output) could be fed into a Beckman pen recorder. In the latter situations, the GM 6020 was used merely as a high-impedance d.c. amplifier providing a signal of suitable voltage and impedance to drive the recorder. The recorder scale was recalibrated to compensate for the interposition of the GM 6020. A voltage stabiliser was included in the mains supply to the recorder but not to the GM 6020, the circuit of which was sufficiently stabilised to be insensitive to mains fluctuations.

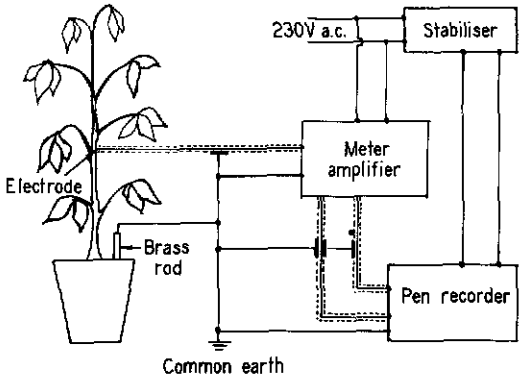


Figure 4. Basic electrical circuit (shown for one internal electrode with reference to earth).

Work on Young Hevea *Seedlings*

The plants were grown outdoors in sand with a daily addition of nutrient solution (BOLLE-JONES, 1956).

K_2SO_4	0.176 g/l
KNO_3	0.101 "
$Ca(NO_3)$ deyhd.	0.328 "
$NaH_2PO_4\ 2H_2O$	0.156 "
$MgSO_4\ 7H_2O$	0.306 "
$(NH_4)_2\ SO_4$	0.198 "
$Fe\ C_6H_5O_7 \cdot 5H_2O$	0.049 "

For electrophysiological work, they were brought into the laboratory just before the experiment. Each pot contained several seedlings *[see Figure 3(b)]*. The following patterns of response were established.

(a) Insertion of a single internal electrode into the stem of the plant and measurement of potential with respect to earth (brass rod inserted in the moist sand of the pot): An immediate rather high negative potential was always recorded. This will be referred to as the 'initial potential' (IP). This potential then fell, rapidly at first and then more slowly, levelling out after several hours to a more or less steady value, typically about 100 mV less than IP *(Figure 5)*. This was interpreted as meaning that the IP is a summation of transient wound potential (WP) caused by insertion of the electrode, superimposed on a relatively steady stem potential (SP). The difference between the initial transient potential and the steady value some hours after electrode insertion should then represent the potential due to wounding (IP—SP = WP) and the 'die-away' shape of the curve should represent the fading away of wound potential as the plant recovered from the electrode insertion. Although this argument is thought to be generally correct, the authors would not claim great accuracy for values of WP calculated in this way, because they depend

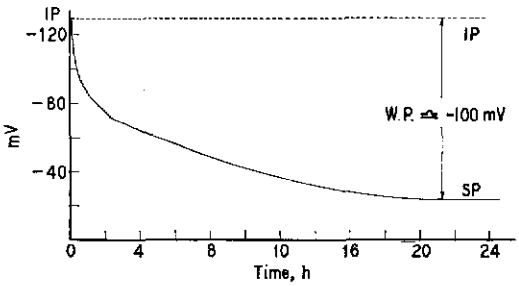


Figure 5. Response of young Hevea *to insertion of an internal electrode. Initial potential (IP) dies away rapidly at first levelling out only after several hours when the recorded potential is the stem potential (SP). WP is the estimated wound potential (IP—SP).*

on an estimate of SP obtained several hours after wounding, and one cannot be sure that these values are truly representative of SP at the time of wounding. They did however indicate a value of WP of the order of —100 mV.

(b) Insertion of an internal electrode in the stem of the plant and measurement of potential with respect to earth followed by wounding the plant near the electrode: In this type of experiment, the insertion of the electrode gave an immediate IP which fell rapidly as described. After an interval of 10 minutes (by which time the potential had completed its initial rapid fall but was still dropping slowly), the stem was pricked 2 mm away from the electrode using the wounding tweezers. An immediate rise in negative potential was observed as a response to the wound. This potential then fell in much the same fashion as does the IP *[Figure 6(a)]*. This was interpreted as meaning that the electrode picked up a wound response signal from the pricking which, for convenience, is called 'response to distant wounding' (RDW). The RDW from such an experiment was always small in comparison with IP peak (it was usually about — 20 to — 30 mV,

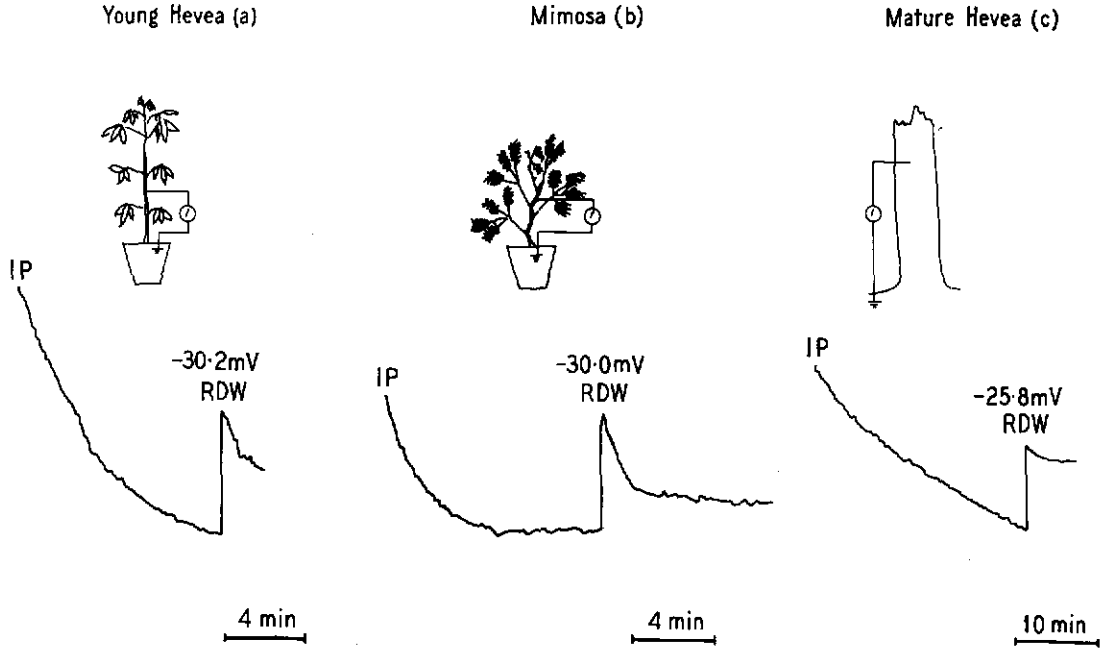


Figure 6. A comparison of response to insertion of an internal electrode followed by wounding 2 mm away from that electrode (pricking). The electrode picks up a response to wounding shown as a spike in the recording. IP—initial potential after wounding; RDW—response to distant wounding.

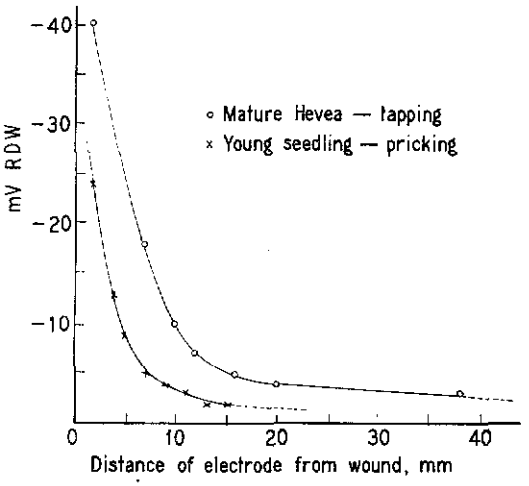


Figure 7. Effect of wounding at different distances from the electrode. The effect dies away rapidly with increasing distance but is detectable a little further from the electrode in the experiment with mature Hevea *where a major wound (tapping cut) was inflicted.*

presumably because signal was lost between the wound and the electrode. To test this idea, a further series of experiments was carried out with wounding further and further away from the electrode. The RDW decreased rapidly with increasing distance and could not be detected at all for a wound 20 mm away *(Figure 7)*. It would seem that electrical potentials occasioned by mechanical wounding of young *Hevea* decay rather rapidly over small distances from a minor wound.

In a third series of experiments, repeated wounds were inflicted in a circle of about 2 mm radius round the electrode. In this case each wound contributed a small peak added to the previous potential so that, after multiple wounding, the combined RDW came much closer to the IP but did not quite reach it *[Figure 8(a)]*. Thus the RDW depends to some extent on the severity of wounding. Taken together, these experiments suggest that, in *Hevea*, there is no mechanism for transmission of a signal of this sort across a large number of cells without losses. These losses could well be power losses, it should be remembered that the instrumentation had an input impedance of 100 mΩ so that a small but finite current was required to activate the instrument (10 μA for a recording of 100 mV) and if the wound excitation could not supply this to the electrode then the reading would fall. Since repeated wounding boosted the reading to near but not above the IP value, it was quite likely that the true wound potential was the same for any wound (of the order of −100 mV) if one could have measured it. But a value approaching this was not recorded unless a sufficiently large number of cells responded at or very near the electrode site. For practical purposes this means that any electrical effects following from wounding in young *Hevea* will always be highly localised near the wound.

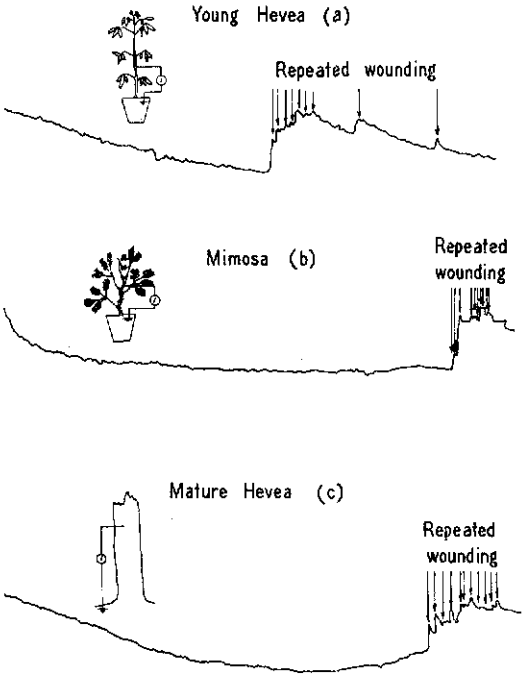


Figure 8. Comparison of the effects of multiple wounding by pricking 2 mm away from the electrode for (a) young Hevea *(b)* Mimosa and *(c) mature* Hevea.

(c) Experiments with two internal electrodes in the stem, and one earth electrode, followed by deliberate wounding: Here each electrode on insertion gave a high negative IP when measured with reference to earth. When sufficient time had elapsed for reduction in the electrode wounding effects, the potential difference (PD) between the electrodes was measured. A small PD was recorded, the upper electrode being positive with respect to the lower. A prick 2 mm from the lower electrode resulted in RDW of the usual form; a transient increase in the recorded PD. After this response had died away, the stem was pricked near the upper electrode. Here the RDW was a transient reduction of PD. In other words the wound always behaves as a generator of negative potential which is added to or subtracted from the measured PD according to the polarity of the electrodes with respect to each other, and the location of the wound *[Figure 9(a)]*.

(d) Experiments with two external electrodes on the stem and one earth electrode followed by deliberate wounding: The two external electrodes were widely separated on the main stem, one near the soil and the other about 30 cm towards the apex. Both showed steady negative potentials with respect to earth (*i.e.*, the IP peak was eliminated because there was no wounding). The upper electrode was negative with respect to the lower one, there being a potential gradient of approximately 7 mV/cm between the two. On wounding (pricking) the stem approximately 2 mm from the upper electrode, the PD between the electrodes showed RDW of the usual form; a transient peaking

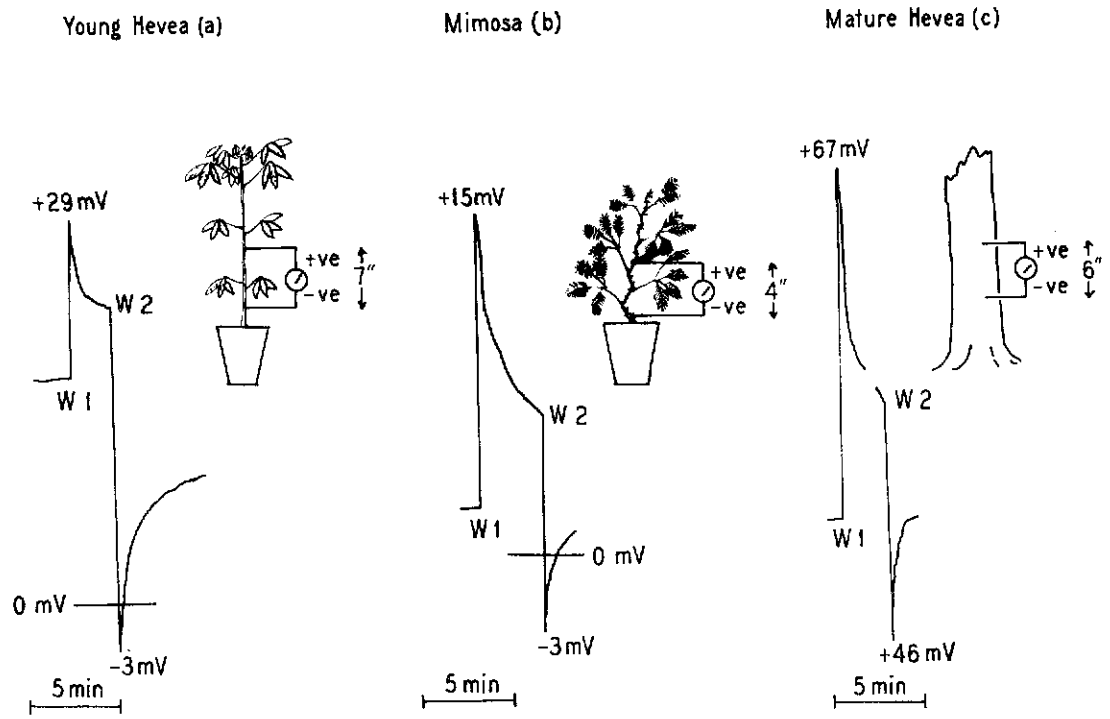


Figure 9. Experiments with two electrodes on the stem of the plant. The recorder indicates a small PD between the two. Wounding near the lower (−ve) electrode (W_1) causes an increase in PD shown as an upward spike in the chart. Wounding near the upper (+ve) electrode (W_2) causes a decrease [going to a reversal of polarity for (a) and (b)] showing as a downward spike.

of about 20 mV dying away as usual and shown as an increase of 'negativity' of the upper electrode (increase in PD between upper and lower electrodes). After this response had died away, a wound was made near the lower electrode. Here again the RDW was of the usual form, shown as an increase in 'negativity' of the lower electrode (decrease in PD between the electrodes). This behaviour is similar to that obtained for the two internal electrodes in the preceding experiments. It seems that the SP and RDW can be detected even without having the electrodes embedded in the tissues. Hence these responses are not apparently due to direct ionic exchanges between the electrode tip and cells damaged by the electrode. Use of external electrodes is of great convenience for work with small plants in that it is not necessary to wait for electrode IP's to die away before the wounding experiments are begun, and the risk of errors from undetected disturbances of the plant causing reopening of the electrode wound is eliminated. These experiments indicate the general electrophysiological patterns covered in this paper, taking young *Hevea* seedlings as the example. This work was repeated with minor variations in technique for mature *Hevea* trees in regular tapping and for *Mimosa pudica*. The variations were as follows:

For work on *Hevea* trees, it was not always possible to transport the full recording equipment to the field and hence some experiments were done by direct readings from the GM 6020 microvoltmeter. Internal electrodes were used, they being easier to work with on trees than on young plants. Because of the much greater bark thickness and the greater volume and pressure of latex it was usual to make a small hole in the bark and insert the capillary of the electrode *(Figure 2)*. The wounding ranged from pricks made in the bark to actual tapping cuts above the electrode.

For work on *Mimosa*, both internal and external electrodes were employed; the latter were more convenient, since internal electrodes caused a 'collapse' from which the plant took hours to recover.

The pattern of response described for the young *Hevea* seedlings was duplicated in every essential aspect by mature *Hevea* trees. The trunk of the tree showed a stem potential with respect to earth. Insertion of internal electrodes gave a high IP, which dropped quickly at first and levelled off over some time. Wounding gave a transient RDW strongly localised near the wound. The magnitude of the wound response calculated from IP–SP was again of the order of −100 mV. The results are shown in *Figures 6(c)*, *7*, *8(c)* and *9(c)*.

The work on *Mimosa pudica* was undertaken mainly to have some standard of comparison using a plant which had been widely studied. Results obtained were broadly similar to those of other workers (UMRATH, 1959 and 1966; SIBAOKA, 1966; DUTT AND GUHATHAKURTA, 1964). The study of *Mimosa* enabled a useful check on the validity of the author's techniques; but perhaps the main interest lies in the comparison between *Hevea* and *Mimosa* for the same sort of measurements.

The patterns of response were remarkably similar to that described for *Hevea* though there were additional phenomena as might be expected from the evident specialisation of this plant. This will be covered in more detail in the *Discussion* and *Appendix B* but, from the experimental point of view, there were really only two major departures from the pattern already described. The first concerns transmission of signal which, in *Mimosa*, could be picked up at considerable distances from the wound. Most writers distinguish three modes of transmission in *Mimosa*, one of which may perhaps be chemically mediated. Nothing approaching such facile, long-distance transmission appears to exist in *Hevea*. The second difference concerns irritability. In *Mimosa*, the authors were able to obtain electrical responses by merely touching a leaf, whereas in *Hevea*, at least a minor wound such as a pin-prick was required. The electrical effects detectable in *Mimosa* go along with turgor changes which produce visible responses from the plant, not seen in *Hevea*. Notwithstanding these special features of *Mimosa*, the similarities of responses with *Hevea* are striking. In both, the stem

potentials were negative with respect to earth, with a gradient along the stem; insertion of an internal electrode gave an IP which died away as described. Subsequent wounding gave a transient response as an increase in 'negativity' of the same form as the RDW in *Hevea*. The WP values for pricking the stem were of about the same magnitude as those detected in *Hevea*, estimated to be of the order of −100 mV. Results are shown in *Figures 6(b)*, *8(b)* and *9(b)*.

Having shown that electrical responses to wounding could be detected in *Hevea*, the next phase of the work was to see whether these wound responses could be modified by subjecting the plant to various physical and chemical treatments.

The first point to be established was whether the wound response could be elicited from dead as well as living tissues.

For this purpose, small portions of the stems of young *Hevea* seedlings were killed by exposing them to a jet of steam for up to 20 minutes, pre-treatment recordings having first been made of IP and RDW for each zone to be steamed. The steaming treatment was localised by insulating the stem with protective paper wrappings except for the small area (approximately 1″ of stem) to be treated. After exposure to steam, the treated zones no longer responded to deliberate wounding and the IP remained at a constant level not showing the usual rapid decline over the first few minutes. There was no recovery and the plant eventually died. This demonstrates that the wound response is associated with the living condition only *(Figure 10)*. It is of interest that, on insertion of the electrode into the steamed area, a steady potential was recorded approximately −100 mV less than the IP previously obtained from the same area before steaming. Assuming that IP = SP + WP and that WP is approximately −100 mV, the implication is that killing the tissues locally eliminates WP but leaves SP still operative.

In treatments with chemicals in which the chemical reduces or eliminates the response, it is necessary to know whether the treatment has killed the tissues or merely suspended their normal wound reaction. Therefore, a quick

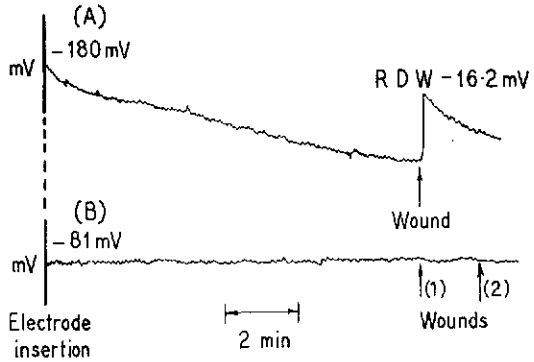


Figure 10. (A) Control experiment with young Hevea *showing normal effect of electrode insertion followed by wounding. (B) shows the effects of steaming the same area for 20 minutes. Neither insertion of the electrode nor wounding gives an inflection on the chart. The level of response from the steamed area is approximately −100 mV less than the peak response due to wounding from the same tissues before steaming.*

criterion of death was needed; this, in a plant, is a difficult question since the plant as a whole can survive the destruction of substantial numbers of its component cells and in any case may show few visible symptoms of death until long after injury. The electrical wound response itself seems to be one of the best criteria for detecting localised death of tissues in a plant. If the treatment used resulted in the permanent elimination of wound response in the particular area where it had been applied, then it was assumed that the treatment had killed tissues in that area. In the young seedlings used for this work such treatments sometimes ended in the death of the whole plant some days later. If the treatment eliminated wound responses only temporarily, it was then considered that the treatment had not killed the tissues over the period of the experiment. In a few cases the wound response recovered after temporary suspension, but later faded out once more, the plant dying eventually. These were regarded as instances of delayed killing, substantial numbers of cells in the treated tissues being still alive up to the time when wound responses were finally lost.

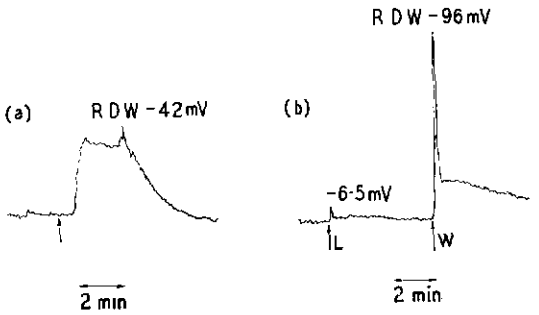


Figure 11. Transmission in Mimosa. *(a) The recording was made using an external electrode with reference to earth. At the time indicated by the arrow a small flame was applied for a few moments to a leaf 3 cm above the cotton wick which was tied around the stem, the rest of the plant was shielded from the flame by cardboard. Approximately ½ min after removal of the flame there was a marked rise in electronegativity at the electrode. (b) The recording was made using an internal electrode with reference to earth, 19 hours after insertion of the electrode. At the time indicated by the arrow L the tip of a leaflet was cut with scissors and about 3 seconds later the leaf drooped at the main pulvinus. The electrode inserted in the stem at the base of the pulvinus picked up a response to leaf drooping shown as a small spike in the recording. On wounding the stem 2 mm away from the electrode (W) (pricking) an immediate response was detected shown as a large spike in the recording. RDW—response to distant wounding.*

Wound potentials in *Mimosa pudica* are considered to be action potentials (potentials arising from depolarisation of active membranes resulting in a temporary change in electrical potential between the resting and stimulated portions of a cell). For *Mimosa* this has been established by techniques including insertion of micro-electrodes into individual cells (SIBAOKA, 1962). For *Hevea* no such direct evidence is yet available, but the responses which have been described so far would be consistent with a similar mechanism. A number of chemical treatments was tried on *Hevea* including chemicals which have been reported to influence action potentials in cells. Most of these reports come from experience in the neuro-physiological field. There are very obvious difference between an animal nerve cell and a plant cell, but where action potentials exist the basic mechanisms are probably similar for all cells. Brief notes on the chemicals chosen are given in *Appendix A.*

For this work internal electrodes were used, recordings being taken with respect to earth after wounding near the electrode. Wounding was by pricking with the tweezer device as near as could be managed to a constant intensity (tweezer spike) and distance (2 mm) from the electrode. Unfortunately the RDW in *Hevea* was so sensitive to wound intensity and distance from the electrode that the magnitude of response was not quantitatively reproducible. Values from −8 to −52 mV have been obtained from individual experiments of this sort. Results therefore need to be interpreted with caution except where the chemical treatment produced a consistently dramatic change (such as a total or near total elimination of response). Several plants were used for each treatment. Usually four electrodes were mounted at the plant in pairs *[Figure 1(c)]*, one pair as near as practicable to the soil, and the other as high up on the stem as possible. Treatments were given by applying cotton wool pads moistened with the chemical or its aqueous solution to the stem in the area where the upper electrode of each pair was to be inserted. The lower electrodes of each pair were used as controls. In the case of ethylene oxide, the plants were enclosed in a polyethylene hood and 10 ml of ethylene oxide in an open dish was placed inside the hood. The hood was removed after one hour (by which time the ethylene oxide had evaporated from the dish).

RDW was measured after treatments of increasing duration unless the first treatment knocked out the response (except in the case of ethylene oxide where only one treatment was given although the effects of this were not immediately apparent). In cases where the treatment greatly affected the wound response, the plant was left undisturbed for 24 hours after which the wound response was again checked to see if recovery had taken place. The results are shown in *Table 1.*

TABLE 1. RECORDINGS OF RDW BEFORE AND AFTER CHEMICAL TREATMENT (ALL POTENTIALS ARE OF NEGATIVE POLARITY)

Treatment	RDW before treatment (mV)	RDW after treatment (mV)	RDW 24 hours after treatment (mV)	Comment
Chloroform applied on cotton wool pads for 30 minutes	Plant (1) a : 19 b : 39 Plant (2) a : 23 b : 14 Plant (3) a : 16 b : 39	a : — b : — a : — b : — a : — b : —	a : 8 b : 30 a : 36 b : 42 a : 9 b : 10	Chloroform temporarily inhibited wound response; this was restored within 24 hours. The plants all survived the treatment.
Di-ethyl ether applied on pads for 30 minutes	Plant (4) a : 10 b : 21 Plant (5) a : 45 b : 23 Plant (6) a : 17 b : 8	a : – b : 4 a : – b : – a : – b : –	a : 23 b : 20 a : — b : 34 a : — b : 18	In plants 5 (apical electrode) and 6 (apical electrode) the treatment seems to have been lethal in the area of application for wound-response was permanently eliminated. In four out of the six experiments the wound response was eliminated or very nearly so, but only temporarily. The plants all survived the treatment.
Ethyl alcohol (commercial) applied on pads for up to 4 hours	Plant (7) a : 24 b : 30 Plant (8) a : 37 b : 15 Plant (9) a : 17 b : 36	a : 33 after 1 hour : 9 after 3 „ : 10 „ 4 „ b : 15 after 1 hour : 9 „ 2 hours : 14 „ 3 „ : 4 „ 4 „ a : 27 after 1 hour : 13 „ 2 hours : 10 „ 3 „ : 3 „ 4 „ b : 17 after 1 hour : 22 „ 2 „ : 4 „ 3 „ : 11 „ 4 „ a : 46 after 1 hour : 16 „ 2 hours : 13 „ 3 „ : 7 „ 4 „ b : 28 after 1 hour : 22 „ 2 hours	a : 24 b : 27 a : 11 6 : 18 a : 11	There were some indications of a temporary reduction in RDW after 3 to 4 hours of application. Patches in the treated areas were discoloured (brown) and dry looking 24 hours after treatment, but all the plants survived.
Procaine hydrochloride applied as 5% aqueous solution on cotton wool pads for 1 hour	Plant (10) 20 Plant (11) 15 Plant (12) 19 Plant (13) 23 Plant (14) 19 Plant (15) 18	12 19 21 24 22 16		No clear indication of any effect at 5% concentration applied for 1 hour. The plants all survived the treatment.
Procaine hydrochloride applied as 30% aqueous solution on cotton wool pads for various periods	Plant (16) a : 10 b : 24 Plant (17) a : 12 b : 25	a : 13 after 1 hour : 15 „ 2 hours : 10 „ 3 „ b : 11 after 1 hour : 21 „ 2 hours : 18 „ 3 „ a : 10 after 1 hour : 14 „ 2 hours : 11 „ 3 „ b : 8 after 1 hour : 16 „ 2 hours : 18 „ 3 „		No clear indication of any effect at 30% concentration applied for periods up to 3 hours. The plants all survived the treatment.

TABLE 1 (Continued)

Treatment	RDW before treatment (mV)	RDW after treatment (mV)	RDW 24 hours after treatment (mV)	Comment
Acetyl choline chloride applied as 10% aqueous solution on cotton wool pads for various periods	Plant (18) a : 39 b : 27	a : 14 after 1 hour b : 12 „ 1 „		No clear indication of any effect at 10% concentration applied for periods up to 3 hours. The plants all survived the treatment.
	Plant (19) a : 30 b : 19	a : 8 after 1 hour b : 19 „ 1 „		
	Plant (20) a : 17 b : 19	a : 26 after 1 hour b : 31 „ 1 „		
	Plant (21) a : 14 b : 27	a : 25 after 1 hour : 14 „ 2 hours b : 32 after 1 hour : 22 „ 2 hours		
	Plant (22) a : 37 b : 45	a : 29 after 1 hour b : 26 „ 1 „		
	Plant (23) a : 12 b : 22	a : 13 after 1.5 hours b : 21 after 1.5 hours : 26 „ 3 „		
	Plant (24) a : 14 b : 21	a : 22 after 1.5 hours : 12 „ 3 „ b : 16 after 1.5 hours : 10 „ 3 „		
	Plant (25) a : 37 b : 15	a : 29 after 1.5 hours b : 11 after 1.5 hours : 9 „ 3 „		
Potassium cyanide applied as 10% aqueous solution on cotton wool pads for various periods	Plant (26) a : 16 b : 22 c : 31	a : 27 after 1 hour : 10 „ 2 hours b : 12 after ½ hour : — „ 1 „ c : — after 3 hours	b : 31	After treatment with KCN for up to 3 hours responses were obtained from some plants but not others. At this stage there were no visible effects on the appearance of the plant. 24 hours later the treated areas showed black patchy discolourations around the previous sites of electrode insertion. In these discoloured patches no wound response could be detected and no latex exuded on pricking in these areas. Elsewhere in the treated area responses were obtained and latex flow was observed. Both plants survived the treatment.
	Plant (27) a : 16 b : 19	a : 10 after 1 hour b : 20 after 1 hour : — „ 3 hours	b : 29 (72 hours). after treatment)	

TABLE 1 (Continued)

Treatment	RDW before treatment (mV)	RDW after treatment (mV)	RDW 24 hours after treatment (mV)	Comment
Potassium ferrocyanide applied as 20% aqueous solution on cotton wool pads for various periods	Plant (28) a : 27 b : 27	a : 17 after 3 hours b : 16 after 3 hours	a : 27 b : 10	There are some indications that potassium ferrocyanide may have temporarily reduced RDW after treatments of some hours (see Plant 29 electrode b). The treatment had no visible effects on the tissues and both plants survived the treatments.
	Plant (29) a : 31 b : 25	a : 14 after 3 hours b : 15 after 1 hour : 4 „ 2.5 hours	a : 18 b : 10	
Potassium ferricyanide applied as 10% aqueous solution on cotton wool pads for up to 4 hours	Plant (30) a : 26	a : 31 after 1 hour : 16 „ 2 hours : 7 „ 3 „ : 5 „ 4 „	a : 15	Potassium ferricyanide appeared to lower the wound response after treatment for 4 hours at a concentration of 10%. All plants survived the treatment and showed normal wound responses 24 hours after treatment.
	b : 22	b : 24 after 1 hour : 12 „ 2 hours : 12 „ 3 „ : 8 „ 4 „	b : 21	
	Plant (31) a : 33	a : 22 after 1 hour : 5 „ 2 hours : 15 „ 3 „ : 2 „ 4 „	a : 22	
	b : 37	b : 7 after 1 hour : 8 „ 2 hours : 5 „ 3 „ : 2 „ 4 „	b : 22	
	Plant (32) a : 14 b : 27	a : 19 after 1 hour b : 25 after 1 hour	a : 17 b : 16	
Glutaraldehyde applied as 6% aqueous solution on cotton wool pads for 10 hours	Plant (33) a : 16 b : 40	a : — b : 44	a : — b : 52	In Plant 33 (electrode a) the treatment appears to have been lethal in the area of application for wound response was permanently eliminated. The wound response was eliminated temporarily in Plants 34 and 35 (basal electrode) but recovery was only limited, responses 24 hours later being comparatively low, probably due to localised death of tissue at the treated zone. All the plants survived the treatment.
	Plant (34) a : 27 b : 30	a : 15 b : —	a : 13 b : 19	
	Plant (35) a : 45 b : 47	a : 26 b : —	a : 2 b : 9	

TABLE 1 (Continued)

Treatment	RDW before treatment (mV)	RDW after treatment (mV)	RDW 24 hours after treatment (mV)	Comment
Ethylene oxide 10 ml allowed to evaporate under polyethylene hood covering plant. Hood removed after 1 hour	Plant 36) 18	29	24 (zero after 5 days with no recovery)	Ethylene oxide produced no obvious change in wound response in 24 hours but the response disappeared permanently in 5 days for Plants (36) and (38). Response in Plant (37) continued for 10 days. All the plants died after the treatment, the symptoms being drooping of the leaves followed by necrosis (blackened tissues) at points of electrode insertion.
	Plant (37) 31	19	25 (52 after 10 days)	
	Plant (38) 28	8	14 (zero after 5 days with no recovery)	
Trichlorophenoxy acetic acid applied as 5% paste on cotton wool pads			Days after treatment 3rd 4th 5th 7th 10th	No clear indication of any effect when applied for up to 2 hours as a 5% paste. All plants appeared normal on 3rd day after treatment. On the 4th day Plant (41) did not yield latex at the treated zone, this was the case on the 5th day for Plants (39) and (40). All leaves had yellowed by the 10th day and following this all three plants gradually died off.
	Plant (39) a : 32	a : 22 after ½ hour : 17 „ 1.5 hours	9 15 14 9 19	
	Plant (40) a : 11	a : 9 after 1 hour : 13 „ 2 hours	12 13 28 23 18	
	Plant (41) a : 19	a : 32 after 1.5 hours : 22 „ 2 „	8 42 17 23 25	

DISCUSSION AND CONCLUSIONS

It has been known for a very long time that, in certain plants, electrical phenomena can be detected following irritation. The first studies seem to have been by BURDON-SANDERSON (1873) on *Dionaea muscipula* (Venus's fly-trap). Subsequently a number of plants have been investigated, especially those which show more or less dramatic movements in response to mechanical stimulation such as the insectivores, and of course *Mimosa pudica*. When Mimosa is stimulated (by touch, injury or electric shock) the stimulus is sensed and transmitted to an effector (the pulvinus) at the base of each leaflet and petiole. The pulvinus contains cells with thin walls; when stimulated, these cells undergo permeability changes which result in a loss of turgor pressure at the base of the leaflets and the petioles, causing them to move as if the plant had wilted.

Very fast adaptations to an environmental change are more often associated with animals than plants, but can obviously occur in both. Action potentials appear to be one common feature to such situations. Following stimulation, this spreads as a propagated potential from one end of the cell to the other and may spread to adjacent cells. It is accompanied by permeability change in cell membranes, which can produce a variety of secondary effects including release of chemical stimulants, which themselves may trigger further action potentials in adjacent cells. This basic mechanism is found in nature in various degrees of specialisation. It is perhaps most highly developed in the animal nerve cell, but is probably a general property of cellular membranes. Most of the detailed information now available on action potentials comes from work with microelect-

rodes inserted into giant nerve cells, such as the nerve axon of the squid. Available giant plant cells for this kind of work are few and not of such an obviously reactive nature. Nevertheless similar work on the very large internodal cells of the fresh-water green algae *Chara* and *Nitella* has demonstrated typical action potentials, mainly in the plasmalemma and, to a much lesser extent, in the tonoplast (NAGAI AND KISHIMOTO, 1964). *Nitella* and *Chara* do not show any visible responses to stimulation except that under the microscope there is a temporary cessation of protoplasmic streaming in the stimulated cells. Action potentials can spread from one internodal cell to another, though this does not always happen. SIBAOKA (1962) was able to insert microelectrodes into individual cells of *Mimosa pudica* and demonstrate action potentials when transmission occurred in the petiole. Elongated parenchyma cells in the protoxylem and phloem seemed to be particularly active.

Excitable membranes (*i.e.*, cell membranes showing action potentials) are always found to be polarised, usually that side of the membrane facing the cell interior (cytoplasm) is around 100 mV negative to the side facing the cell exterior (in nerves typically about 90 mV, in plant membranes figures of from 5 to 160 mV have been reported for different types of membrane, see UMRATH, 1959).

The authors' interest in this field arose initially from a study of the properties of the latex vessel of *Hevea brasiliensis* in relation to flow of latex after tapping. In the course of this work it became clear that leakage through certain sub-cellular membranes could be important and that any explanation of observed flow patterns based on this would have to postulate that these membranes leaked as a localised response to wounding (SOUTHORN AND EDWIN, 1968; SOUTHORN, 1968.). Subsequently, circumstantial evidence was found for electrical polarisation of the membranes concerned, and the authors began to look for mechanisms which might assist in causing leakage of a polarised membrane as a wound response. The analogy with *Mimosa* was too striking to be ignored. In *Mimosa*, wounding results in a rapid transfer of material across cellular membranes, resulting in visible movements of petioles and leaflets. Could it be that in *Hevea*, wounding might induce changes in polarised membranes near the wound, so giving rise to effects within the latex vessel? At this stage electrical response to wounding had not been looked for in *Hevea*.

The present work shows that when *Hevea brasiliensis* is wounded, electrical responses are produced which exhibit similarity in polarity, magnitude and duration to those observed in *Mimosa pudica*. These responses are transmitted over small distances but are much more localised than in *Mimosa*. They arise only from living tissues. Some chemicals (chloroform and ether) can temporarily inhibit the electrical responses; both are anaesthetics which, in animals, block conduction or transmission of nerve impulses. Both have been reported to lower plant potentials (LUND, 1926 and 1928). Since the action of these treatments was temporary, one could be sure that loss of electrical response was not due to death of the tissues.

Demonstration of electrical wound responses in *Hevea* does not of course prove the hypothesis that they contribute to the blocking mechanism, but their existence now makes this idea worth serious consideration. If correct, one would expect treatments with anaesthetic reagents such as chloroform and ether to exert at least a temporary yield stimulation effect in circumstances where plugging is a major factor in latex flow. Since this work was completed, there have been reports (BANCHI, 1967) of field trials with chloroform in which such a stimulatory effect is claimed. However it is intriguing why known yield stimulants like 2,4,5-T did not affect the wound potential.

Apart from the possible direct bearing of this study on latex flow research, it is of interest that *Hevea brasiliensis* has been shown, for the first time, to exhibit definite patterns of electrical response to wounding though WHITE (1946) considered the possibility of its existence when he studied the effect of tapping on tissue resistance. Electrical responses are likely to be associated with transfer of materials across membranes leading to subtle changes in cell physiology. Thus, the rubber tree has a fast 'awareness-to-wounding' mechanism available.

However one cannot say for certain at this stage the exact use made of it. It may well be relevant to latex flow.

ACKNOWLEDGEMENT

Thanks are due to Mr. Yee Shin Meng and Mr. Chew Sew Wan for their technical assistance.

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APPENDIX A — CHEMICALS USED

Some of the chemicals used in the experiments have already been shown to have an effect on electrical potentials in plants. LUND (1926) reported reversible inhibition of electrical polarity in the stem of *Obelia* produced by cyanide, ether and chloroform. CLARK (1938) reversibly depressed electrical potential across sections of the coleoptile of *Avena* after exposure to ether. BOSE (1928) reported a reversible depression of excitability in *Mimosa* by ether and chloroform.

Certain other chemicals were considered to be of interest in this connection in *Hevea*, possibly influencing the electrical behaviour of the tissues.

Trichlorophenoxyacetic Acid

Trichlorophenoxyacetic acid is a well established latex yield stimulant in *Hevea* for which the mechanism of action is not known although several possibilities have been recognised including effects on membrane permeability (BLACKMAN, 1961).

Ethylene Oxide

Ethylene oxide has been reported by TAYSUM (1961), to greatly prolong latex flow time after tapping in *Hevea*. The latex was stable, remaining liquid at 15°C for long periods if kept sterile.

Glutaraldehyde

Glutaraldehyde—a commonly used tissue fixative, changes the structure of lipid membranes and presumably therefore alters permeability properties also.

The ionic movements giving rise to a transitory variation of electrical potential as a response to wounding in *Hevea* might be comparable to those giving rise to the action potential of certain membranes in other tissues. From the neuro-physiological point of view, anaesthetic agents are capable of reversibly blocking the conduction or transmission of nerve impulses but as yet there is no satisfactory answer to the problem of their mode of action. Anaesthetic drugs produce their effects primarily by modifying the responses of the Central Nervous System (CNS) including depression of the sensory and motor functions. Anaesthetics also act on peripheral receptors, the neuro-muscular junction and autonomic ganglia and probably therefore reduce the afferent input to the cortex and at the same time render the cortex unable to make effective use of the afferent input. Some of the theories of anaesthesia are concerned with the correlation of anaesthetic action and certain physical properties of the agents such as their high lipid solubility. Others deal with the ability of the drugs to depress the actions of various enzyme systems (cellular narcosis occurs before any demonstratable change in oxygen uptake). A further possibility is that the drugs produce their effect by interrupting synaptic transmission of impulses within the CNS (ROOT AND HOFMANN, 1963 and 1965; WOOD-SMITH AND STEWARD, 1964).

Chemicals of interest in these connections are:

Diethyl ether. Diethyl ether markedly reduces skeletal muscle tone and this is due mainly to depression of transmission at the neuro-muscular junction—by competing with acetyl choline, released from the nerve terminal, for the active sites on the muscle end-plate membrane so preventing its depolarisation.

Ether may also affect the utilisation of ATP (INESI *et al.*, 1967). Rapid inhibition of NADH oxidation in heart muscle preparations by ether has been reported (KANIUGA *et al.*, 1968).

Chloroform. Primary action on the CNS.

Alcohol. Alcohols are depolarising agents. In brain cortex slices, alcohols depress the P/O ratio and reduce ATP formation by the suppression of NADH oxidation and its associated phosphorylations. It seems probable that alcohols associate with a phospholipid group in the membrane structure which is linked with oxidative metabolism.

Procaine. Procaine is a local analgesic preventing conduction of the nerve impulse by action on nerve trunks, on synapses, on sensory endings, or at myoneural junctions. Local analgesics do not cause depolarisation of the nerve membrane but the block seems to involve

stabilisation of the usually labile nerve membrane so preventing the ionic movements necessary to the propagation of impulses. Procaine produces mild blockage of transmission at autonomic ganglia and myoneural junctions. At both sites the block is probably in part due to depression of acetyl choline release and partly a non-depolarising competitive block. Smooth muscle is depressed by direct action.

Acetyl choline. Acetyl choline is the chemical transmitter of nerve impulses at several sites including motor nerve endings to skeletal muscle, where it depolarises the motor endplate by breaking down the selective permeability properties of the membrane.

Cyanide. Cyanide blocks the normal metabolic sources of ATP synthesis, a supply of ATP essential for active transport of material across membranes and maintenance of polarisations.

Ferrocyanide. Crab nerves treated with ferrocyanide lose K^+ and gain Na^+ which may account for their depolarisation, or may be the result of direct reaction by ferrocyanide with membrane material (LIEBERMAN *et al.*, 1967.)

APPENDIX B — *MIMOSA*

In *Mimosa*, the pulvinus offers a barrier to transmission of signal which may be sufficient to block it completely if the signal reaching the pulvinus is weak, or to impose a variable delay depending partly on the magnitude of the stimulus. Thus a very gentle touching of the leaflets may collapse a pair of leaflets, or even a single leaflet and go no further. A slightly more vigorous touch may collapse several pairs of leaflets with a just detectable delay between the collapse of each pair. Touching the petiole brings about collapse of the leaf at the main pulvinus where the leaf stalk joins the main stem. If the tip of a leaflet is cut with scissor then the leaf reacts in one of three ways:

(i) all the leaflets in the vicinity collapse but the reaction goes no further;

(ii) the whole leaf petiole droops from the main pulvinus within 1–3 seconds after cutting;

(iii) the leaf droops at the main pulvinus but after a much longer delay than in (ii), *i.e.*, 20–30 seconds after cutting.

More vigorous stimuli such as burning or crushing will produce a widespread reaction which may go through the whole plant taking many seconds to reach leaflets remote from the wound. The literature on this subject is somewhat confused but most authors distinguish three modes of propagation, fast, moderately fast and slow. The slowest of them, produced by wounding stimuli of burning or crushing may be mediated by the release of a chemical excitor (BONNER AND ENGLISH, 1938; ENGLISH *et al.*, 1939; UMRATH, 1959). It shows a negative electrical potential irregular in shape and long in duration (of the order of −40 to −50 mV in these studies), see *[Figure 1(1a)]*.

The most rapid conduction occasionally observed in the leaf following a cutting stimulus has been investigated by UMRATH (1966). Electrical recordings from the petiole demonstrated a brief negative deflection of up to 10 mV if a sub-petiole was cut through which was interpreted as an action potential.

The moderately rapid conduction, which is also by means of an action potential, is produced everywhere in the leaf and stem by every sort of stimulus.

Cells in the pulvini of *Mimosa* respond electrically and mechanically when the action potentials generated in the petiole or pinna-rachis reaches them. The cells in the pulvini (effector mechanism) show action potentials of shorter duration than those recorded in the petiole (conductor mechanism). The mechanical response (leaf drooping) follows about $\frac{1}{10}$ to $\frac{1}{20}$ second after the electrical response (DUTT AND GUHATHAKURTA, 1964; SIBAOKA, 1966).

Responses for *Mimosa* have been reported of around −100 mV and this sort of value is obtained in our experiments either by the difference between the initial response from an internal electrode and the steady stem potential when the effect of wounding has died away, or alternatively by wounding some distance away from an external electrode. The second condition contrasts with *Hevea* where

the response to distant wounding is always much less than −100 mV depending on the distance of the wound from the electrode (normally −20 to −30 mV for a wound 2 mm away). The transmission of response is thus very much more effective in *Mimosa* than in *Hevea*. The transmission mechanism in *Mimosa* is obviously complex.

DISCUSSION

Chairman: Prof. G. E. Blackman

Mr. S. W. Pakianathan suggested that the effects of either an electrical charge due to wounding or a change in osmotic concentration would not be strictly localised near the tapping cut where plugging occurred. Dr. Southorn said that the experimental evidence showed that electrical effects in *Hevea* were strongly localised. Depolarisation was one among several mechanisms which might cause lutoids to leak (as also was osmotic shock); he agreed that osmotic changes would not be localised. This was one reason for the interest in localised effects such as electrophysiological responses.

Prof. J. Bonner asked what changes would be expected in the pattern of latex flow if there were no lutoids, due to deletion of the genes for their formation. Dr. B. L. Archer said that some particles intimately associated with the lutoids, or perhaps even the lutoids themselves seemed to be responsible for the reduction of HMG-CoA to MVA. Therefore, deletion of the lutoids might terminate biosynthesis of rubber. He agreed with Mr. G. F. J. Moir that difficulties in separating the particles present in the 'bottom fraction' had so far prevented definitive determination of the role of lutoids in biosynthesis. Dr. Southorn added that if the lutoid was, in plants, the homologue of the animal lysosome, it was probably an organelle essential for the life of the cell irrespective of any function it had in the biosynthesis of rubber.