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The Application of a New Push-Pull Amplifier in Plant Electrophysiology

Zastosowanie nowego typu wzmacniacza przeciwsobnego w elektrofizjologii roślin

Применение нового симметрического усилителя постоянного тока в электрофизиологии растений

The achievements made in Electrophysiology are undoubtelly connected with the development of adequate experimental methods especially those which made possible investigations of bioelectrical parameters and of cells and tissues which accompany excitation.

The amplitude of impulses of action currents in organisms ranges from very few microvolts to approximately two hundred millivolts. The duration of impulses varies also in a wide range from one millisecond to several seconds in plants. The frequency of oscillations of bioelectrical currents does not usually exceed several hundred cycles per second, but very slow bioelectrical processes lasting for several minutes are characteristic of some cells and organisms. Investigation of such processes is possible with the application of very stable d. c. amplifiers with a small zero drift and a high amplification. Unfortunately commercial amplifiers which would satisfactorily fulfil such requirements are not at present available in this country.

The above mentioned difficulties are more pronounced in the field of Plant Electrophysiology where an additional restriction is imposed on the measuring technique, namely the high electrical resistance of plant tissue which is of the order of 10^5—10^6 ohms [6]. The high resistance of plant tissue involves a high input impedance of the measuring device, which should be not less than 10^8 ohms.

However, present electrophysiological experiments involve the application of glass microelectrodes with the tip diameter of ca. 1 μ. The resistance of such a microelectrode is as high as 10—30 Mohms, electrical measurements thus being only possible with the use of a very high input impedance d. c. amplifier. This follows from the fact that the resistance of such a microelectrode in series with the input resistance of the amplifier would create a voltage divider diminishing the amplitude of the impulse transmitted to the real input of the amplifier (Fig. 1).

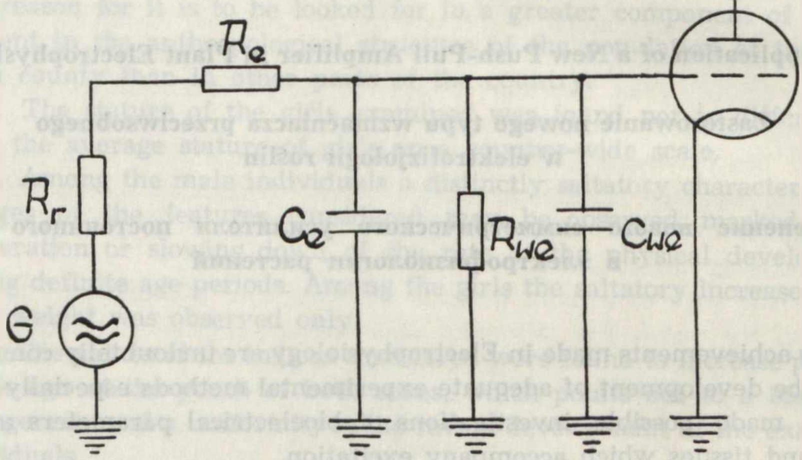

Fig. 1. Equivalent circuit of the input of the amplifier

As far as the Electrophysiology of plants is concerned the experimentalist has to face an acute lack of suitable commercial electronic apparatus. The goal of the present paper is to cover at least partly the great need for the development of electrophysiological technique in Plant Physiology in Poland.

APPARATUS

Measurements of bioelectrical potentials are usually carried out by means of direct current amplifiers of different types, though most often simple amplifiers with direct coupling between particular stages are used [1, 8, 17]. A big disadvantage of such circuits is a high zero drift which either largely limits the sensitivity of the device or does not allow the carrying out of long term experiments.

The amplifiers, working on the principle of the change of direct current into alternating with a relay at the input of a low frequency amplifier, possess a relatively small zero drift, but the frequency range, which is determined by relay is so narrow (e. g. 10—8 cycles — 15, 17), that only very slow potential changes can be amplified.

It is perhaps noteworthy that sometimes very simple d. c. bridges with electrometric valves are used for resting potential measurements, their sensitivity being even as high as a hundred microvolts per one division of the galvanometer scale with the zero drift not exceeding 0.2 mV per hour (11, 12).

We must now discuss the experimental conditions referring to both the measurements of bioelectrical potentials and the features of amplifiers used for this purpose.

1. Input impedance. A very high input impedance of the measuring device is necessary due to the high resistance of the tissue (which in the case of plant tissue is as high as 16^6 ohms per cm^2) as well as the enormously high resistance of microelectrodes transmitting current to the input of amplifier.

The problem of grid current is in fact identical with that of the high input impedance but in view of the possible effect of this current on the tissue it deserves special attention. It appears from the literature and our own experience that in the case of plant tissues this current should not exceed the value of 10^{-10}A. Such a value can be safely considered to be negligible and not affecting cells and therefore the recorded potential difference.

Strictly speaking in this case one can neglect the changes of grid potential occurring at the moment when relatively small resistance is connected to the input. The resistance in question is that of plant tissue (10^6 ohms), which is small enough compared with the input impedance (10^{11} ohms) of the amplifier.

Sensitivity. The maximum sensitivity of the amplifier is determined by the range of the measured potential differences. The amplitudes of resting potentials reach the values of 100—200 millivolts, but the action current pulses amplitude range is very wide starting from several tenth of microvolts, the time of duration of the rising phase being only of the order of milliseconds .

3. Zero drift. The zero drift in d. c. amplifiers is brought about by unsteadiness of the parameters connected with valves and power supply. The voltage appearing at the amplifier output due to the drift is added to the recorded potential difference thus causing the error, which may be quite substantial. Among many types of amplifier circuits described in the literature there is one in particular with quite a high

degree of stability, namely the parallel bridge circuit (4). The signal is amplified in both arms of the bridge in opposite phases, but the voltage changes caused by the drift and floating remain in the same phases and therefore they are not detectable by the measurihg device (oscilloscope).

4. Frequency range of amplifiers. The time of the rising phase of the voltage impulses produced by living cells may reach several microseconds, so the range of transmitted pulses should be as large as to range from zero to more than ten kilocycles per second. This range is mainly limited by a high time constant of the input equivalent circuit shown in Fig. 1.

The tissue is regarded as a generator G with internal resistance R_r. The values R_e and C_e denote the resistance and the capacity of a measuring microelectrode (electrode). R_{in} and C_{in} are correspondingly the input resistance and the capacity. The time constant of the input circuit is expressed by the following simple equation;

$T = R \cdot C$, where

and $C = C_e + C_{in}$.

If $C = 50$pF (which is relatively easily reached) and $R_r = 10^7\ \Omega$, $R_{in} = 10^{11}\Omega$, the time constant $T = 100$ microsec. In some cases this value may still be too high. A fairly high compensation can be achieved by means of a positive feedback, the last value being dependent upon frequency (10, 11).

5. Noise. Another factor which limits the sensitivity is noise. In electronic valve devices there are three main sources of this phenomenon; a) the thermal noise of the grid resistance, b) the noise of grid current and c) the noise of the emission current. As in the case when receiving valves are used as electrometric ones the noise of the emission current is especially high because then there is necessity for cathodes with a low heating level.

The above briefly described conditions were taken into account, so the amplifier constructed by us satisfies all the requirements and may be used for bioelectrical potential measurements both on plant and animal cells and organisms. The general applicability of the amplifier is due to the fact that it is based on the combination of the advantages of the two types of amplifiers, which are as follows: a) the amplification of very slow potential changes of the order of millivolts by means of the d. c. amplifier, with the characteristic frequency starting from zero, b) the amplification of the action currents with the amplitudes of several microvolts by the a. c. push-pull amplifier without zero drift.

The d. c. amplifier operates in the double sided push-pull amplifier circuit which seems to be the most suitable to the amplifiers with direct coupling, where an additional restriction the required sensitivity ap-

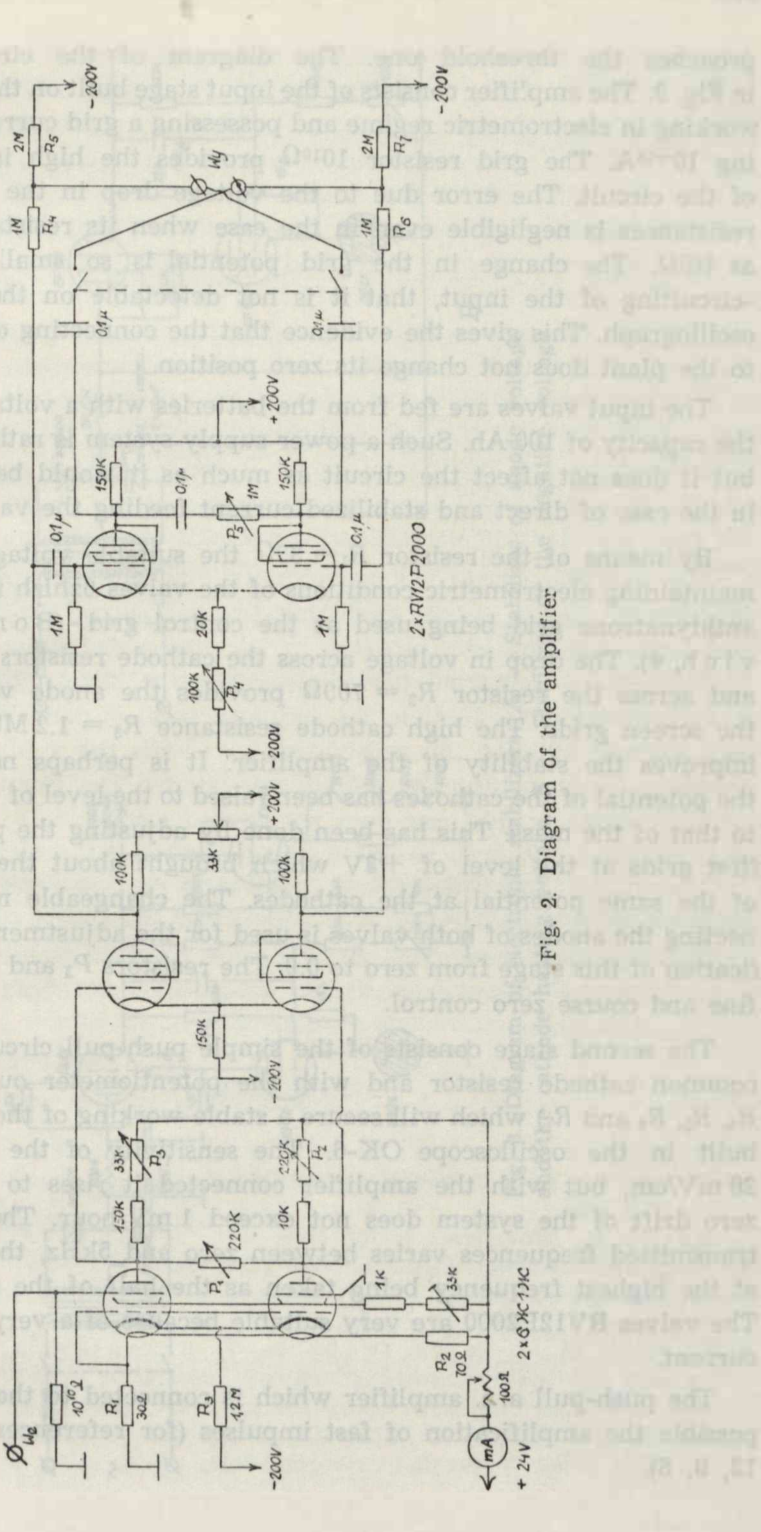

Fig. 2. Diagram of the amplifier

proaches the threshold one. The diagram of the circuit is shown in Fig. 2. The amplifier consists of the input stage built on the valves 6zhlzh working in electrometric regime and possessing a grid current not exceeding 10^{-14}A. The grid resistor $10^{10}\Omega$ provides the high input resistance of the circuit. The error due to the voltage drop in the microelectrode resistances is negligible even in the case when its resistance is as high as $10^{7}\Omega$. The change in the grid potential is so small after a short--circuiting of the input, that it is not detectable on the scope of the oscillograph. This gives the evidence that the connecting of the amplifier to the plant does not change its zero position.

The input valves are fed from the batteries with a voltage of 24V. and the capacity of 100 Ah. Such a power supply system is rather complicated but it does not affect the circuit as much as it would be affected even in the case of direct and stabilized current feeding the valves.

By means of the resistor $R_1 = 30\Omega$ the suitable voltage required for maintaining electrometric conditions of the valves 6zhlzh is achieved, the antidynatrone grid being used as the control grid (Bonch-Bruyevich, 4). The drop in voltage across the cathode resistors in both valves and across the resistor $R_2 = 700\Omega$ provides the anode voltage also for the screen grids. The high cathode resistance $R_3 = 1.2\,M\Omega$ substantially improves the stability of the amplifier. It is perhaps noteworthy that the potential of the cathodes has been raised to the level of +3V compared to that of the mass. This has been done by adjusting the potential of the first grids at the level of +3V which brought about the establishment of the same potential at the cathodes. The changeable resistor P_1 connecting the anodes of both valves is used for the adjustment of the amplification of this stage from zero to 0.9. The resistors P_3 and P_2 are used for fine and course zero control.

The second stage consists of the simple push-pull circuit with a high common cathode resistor and with the potentiometer output (resistors R_4, R_5, R_8 and R_7) which will secure a stable working of the d. c. amplifier built in the oscilloscope OK-6. The sensitivity of the oscillograph is 20 mV/cm, but with the amplifier connected it rises to 3 mV/cm. The zero drift of the system does not exceed 1 mV/hour. The range of the transmitted frequences varies between zero and 5kHz, the amplification at the highest frequency being taken as the half of the maximum one. The valves RV12P2000 are very suitable because of a very small heating current.

The push-pull a. c. amplifier which is connected to the output makes possible the amplification of fast impulses (for references see literature 13, 9, 5).

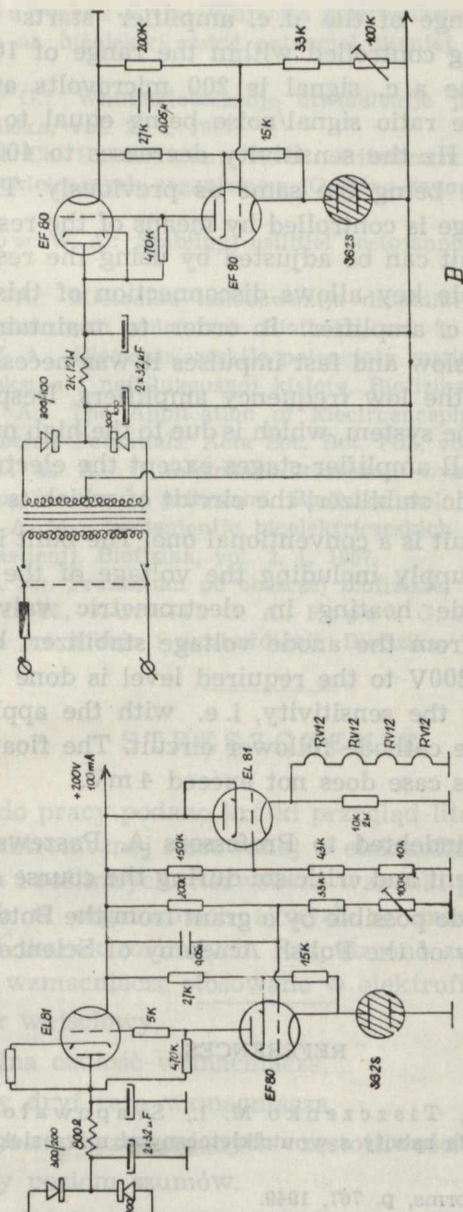

Fig. 3. Diagram of electronic stabilizers A — Stabilizer of anode voltage and the cathode heating voltage, B — Stabilizer of the negative voltage

The frequency range of the d. c. amplifier starts from 10 Hz, the upper part of it being controlled within the range of 100 Hz to 10 kHz. The sensitivity of the a. c. signal is 200 microvolts at the frequency range 10—100 Hz, the ratio signal/noise being equal to 5. For the frequences 10 Hz to 10 Hz the sensitivity decreases to 400 microvolts/cm, the ratio signal/noise being the same as previously. The amplification at this frequency range is controlled by means of the resistor P4 and the highest frequency limit can be adjusted by using the resistor P_5 (100 Hz to 10 kHz). The double key allows disconnection of this amplifier from the output of the d. c. amplifier. In order to maintain correct phase proportions between slow and fast impulses it was necessary to cross the input and output of the low frequency amplifiers. Despite this there is no self-induction in the system, which is due to the high output resistances (R_4, R_5, R_8 and R_7). All amplifier stages except the electrometric one are fed from the electronic stabilizer, the circuit of which is shown in Fig. 3.

The stabilizer circuit is a conventional one. The built in millivoltmeter controls the power supply including the voltage of the batteries which are used for cathode heating in electrometric valves. The valves RV12P2000 are fed from the anode voltage stabilizer, but the decrease in the voltage from 200V to the required level is done in a very useful way without altering the sensitivity, i. e. with the application of EL81 valves working in the cathode follower circuit. The floating level of the supply voltage in this case does not exceed 4 mV.

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STRESZCZENIE

We wstępie do pracy podano krótki przegląd literatury odnoszącej się do aparatury elektronicznej stosowanej w elektrofizjologii komórek i tkanek zwierzęcych i roślinnych. Rozważono warunki pomiarów potencjałów bioelektrycznych w organizmach żywych ze szczególnym uwzględnieniem komórek i tkanek roślinnych. Rozpatrzono zasadnicze cechy, które winny posiadać wzmacniacze stosowane w elektrofizjologii; są to:

a) duży opór wejściowy,
b) maksymalna czułość wzmacniacza,
c) minimalny dryf zera wzmacniacza,
d) szerokie pasmo wzmacnianych częstotliwości,
e) minimalny poziom szumów.

W artykule niniejszym opisano skonstruowany przez autorów nowy uniwersalny wzmacniacz przeciwsobny odpowiadający wymienionym warunkom najlepiej ze wszystkich stosowanych dotychczas wzmacniaczy w badaniach elektrofizjologicznych i opisanych w literaturze.

Podjęcie niniejszej pracy było konieczne ze względu na brak aparatury elektronicznej, którą można byłoby stosować w elektrofizjologii roślin.

РЕЗЮМЕ

Во введении к настоящей работе дается краткий литературный обзор по электронным усилителям применяемым в электрофизиологии клеток и тканей животных и растительных организмов. Рассматриваются условия проведения измерений биоэлектрических потенциалов в живых организмах, причем особое внимание обращается на растительные клетки и ткани.

Определены основные свойства которыми должны обладать усилители предназначенные для электрофизиологических исследований: а) большое входное сопротивление, б) максимальная чувствительность, в) минимальный дрейф нуля, г) широкий диапазон усиливаемых частот, д) минимальный уровень шумов.

Поскольку до сих пор не было усилителей которые удовлетворяли бы всем этим требованиям авторами создан и описан новый универсальный усилитель постоянного и переменного тока, превышающий по качеству все известные усилители применяемые в электрофизиологических исследованиях.

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