plasma (pooled values) there was significantly ($p < 0.01$) more PGE than PGF. Some variation in PG levels was found following storage of some of the plasmas for 7 weeks and in these cases, both values were discounted.

Discussion. The lack of differences in primary prostaglandin levels in the plasma in women of different body constitution and of different nutritional status lends little support, per se, to the prostaglandin hypothesis of obesity[7]. This result showing a significantly higher plasma concentration of PGE than PGF is at variance with a previous study when greater amounts of PGF than PGE were observed in human serum[11]. However, the absolute levels of PGE in our samples are compatible with previous observations[12]. Some of the discordant results of different studies may be explained by the presence of a PG-metabolizing system in plasma which reduces, significantly, plasma levels of PG within min in vitro[13]. This system of PG degradation plus that of the lungs and other vascular beds known to operate[14], indicate that elucidation of any role for prostaglandins, in vivo, must be accompanied by rapid removal of blood, and rapid 'fixing' of the blood sample in order to minimize PG degradation.

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PRO EXPERIMENTIS

A micro-electrode amplifier with an infinite resistance current source for intracellular measurements of membrane potential and resistance changes under current clamp

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Summary. A microelectrode amplifier for intracellular electrophysiological research is described. It is equipped with an electronic infinite resistance constant current source for the injection of current into biological cells. With this amplifier the potential changes, the resistance changes and the dependence on extrinsic current of single cells can be measured independently and simultaneously.

Investigators of invertebrate visual sensory cells are repeatedly faced with the need to measure simultaneously the membrane potential of single cells, their resistance changes, and the dependence on extrinsic current (current clamp) of these membrane properties.

The latter types of measurements are carried out with a single intracellular microelectrode by extending the microelectrode amplifier for the membrane potential measurements with a current source in a bridge circuit arrangement. The drawbacks of the currently applied circuits and an improved design are discussed in this note.

The classical bridge circuit[2] for measuring resistance changes is given in figure 1. The membrane potential is measured through the microelectrode (with resistance R_e) and the follower amplifier A_1. Current is sent into the cell by feeding a large resistor R_s with a potential E_s (DC for current clamp and AC square waves for resistance measurements). The voltage drop over the electrode resistance (R_e) and the membrane resistance (R_m) is balanced by the output ($-E_sR_2/R_1$) of amplifier A_2. The bridge is in balance when

$$R_2/R_1 = (R_e + R_m)/R_s. \quad (1)$$

The changes in the membrane resistance (R_m) upon illumination appear as an imbalance of the bridge.

In the usual Wheatstone bridge the output is measured differentially, and the input of the bridge is fed single-ended whereas in the circuit of figure 1 the bridge output is measured single-ended, and the input is fed differentially.

The circuit of figure 1 suffers from several drawbacks.

1. The potential measured is not the membrane potential V_m but a potential V_o via the potential divider consisting of ($R_e + R_m$) and R_s, and the gain of amplifier A_2 which equals unity to a very good approximation:

$$V_o = \frac{R_s}{(R_s + R_e + R_m)} \cdot V_m \quad (2)$$

with $R_s = 1000$ MΩ and $R_e + R_m = 100$ MΩ (typical values); this means a systematic error of 9%.

2. The cell is loaded. With R_s grounded, $V_m = -60$ mV and $R_s + R_e + R_m = 1100$ MΩ a current of 55 pA is loading the cell which is much more than the input current of A_1 (< 1 pA).

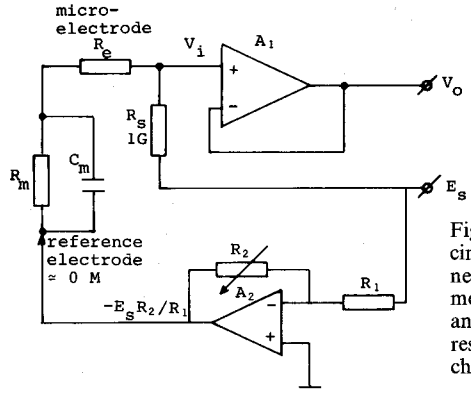

Fig. 1. Classical bridge circuit for simultaneously measuring the membrane potential and the membrane resistance (R_m) changes of single cells.

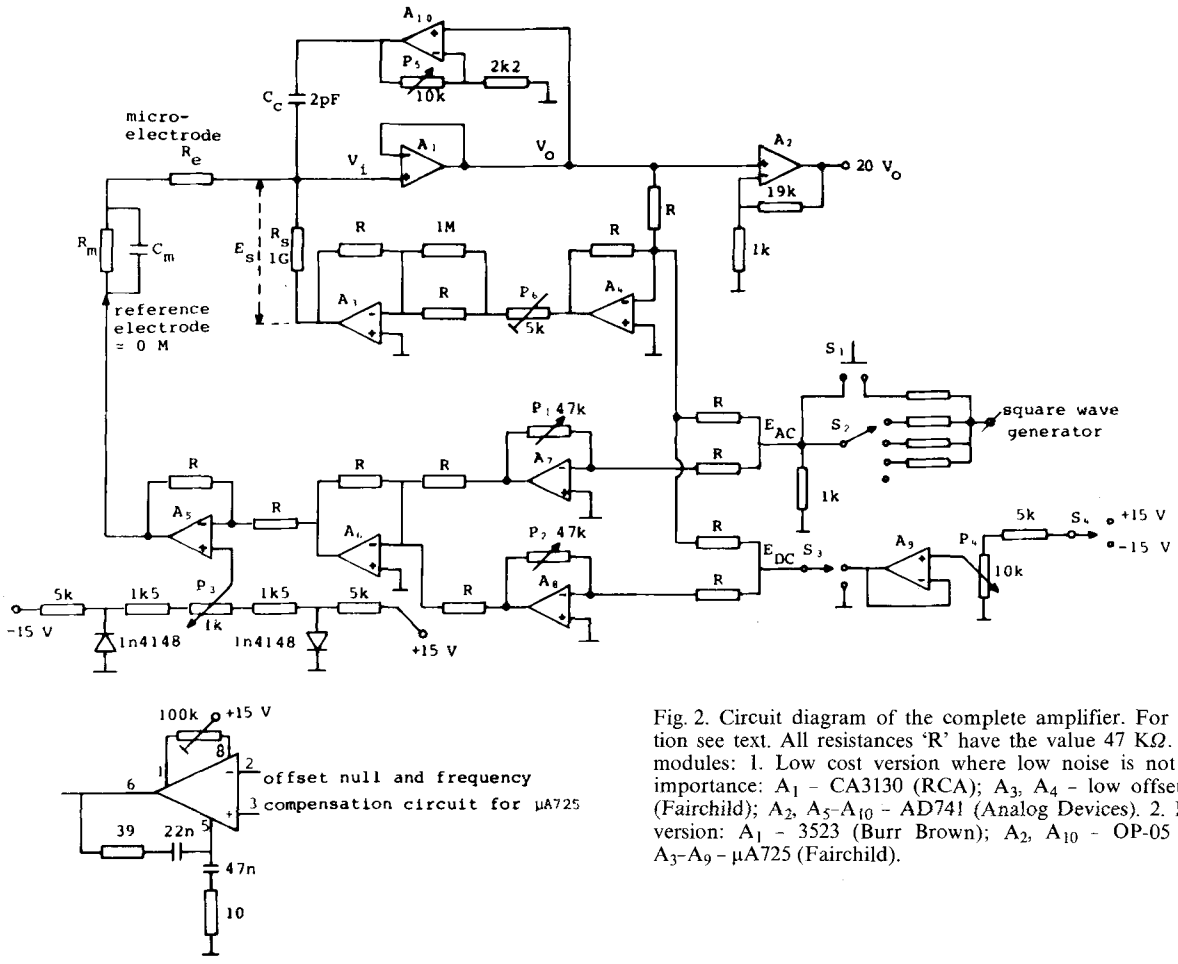

Fig. 2. Circuit diagram of the complete amplifier. For a description see text. All resistances 'R' have the value 47 KΩ. Amplifier modules: 1. Low cost version where low noise is not of prime importance: A_1 - CA3130 (RCA); A_3, A_4 - low offset - µA725 (Fairchild); A_2, A_5-A_{10} - AD741 (Analog Devices). 2. Low noise version: A_1 - 3523 (Burr Brown); A_2, A_{10} - OP-05 (Bourns); A_3-A_9 - µA725 (Fairchild).

3. Resistance measurements under current clamp cannot be executed easily, because the electrode resistance is nonlinear; its slope and chord resistances are equal at zero current only. Under current clamp the chord resistance has to be used in equation (1), meanwhile for resistance measurements the slope resistance has to be applied.

The principle of bootstrapping solves the 1st and 2nd problems. The potential source E_s is replaced by a source (E_s+V_o) where V_o is the output of amplifier A_1 (figure 1 and 2). Amplifiers A_3 and A_4 together create this source: A_4 adds E_s and V_o, A_3 corrects the sign. The potential over R_s is now:

$$V_s = E_s + V_i g_1 g_3 g_4 - V_i \quad (3)$$

where g_1, g_3 and g_4 are the gains of amplifiers A_1, A_3 and A_4 respectively, and V_i is the potential at the input of A_1. The current I_s through R_s (and thus also through R_e and R_m) is

$$I_s = \{E_s + V_i(g_1 g_3 g_4 - 1)\}/R_s \quad (4)$$

For $g_1 g_3 g_4 = 1$ (fine adjusted by P_6) $I_s = E_s/R_s$ which is independent of R_m, R_e and V_m. The apparent value R_s^* of R_s is

$$R_s^* = R_s/(1 - g_1 g_3 g_4) \quad (5)$$

which goes to infinity for $g_1 g_3 g_4 = 1$.

The loading of the membrane is dependent on the joint offset potentials of A_1, A_3 and A_4. With the opamps used it can be kept effortlessly below 10 mV, which means a loading current below 10 pA for $R_s = 1000$ MΩ. In our circuit we measured $1/(1 - g_1 g_3 g_4) > 100$ and $V_{offset} < 2$ mV. The last drawback is overcome by balancing separately for AC currents with P_1 in A_7 and with P_2 in A_8 for DC currents. In this way E_s is split in E_{DC} and E_{AC} (figure 2).

The values of the resistances associated with switch S_2 are chosen so that the resulting magnitudes of the square waves at the variable contact of S_2 are respectively 1, 0.5 and 0.2 V peak to peak. Together with the current injecting resistor (1000 MΩ) this amounts to 1, 2 and 5 MΩ/mV respectively at the output of A_1.

By pressing S_1 (briefly) a square wave current of 10 nA peak to peak appears at the input of A_1 which facilitates penetration of the cells[3]. The amount and sign of direct current injected into the cell is controlled by S_3, P_4 and S_4. The outputs of A_7 and A_8 are added in A_6 and provided with the correct sign by A_5.

To buck out all unwanted potentials A_5 is supplied with a variable potential at the + input (P_3). The amount of capacitance compensation (via A_{10} and C_c) is set by P_5. Though a mains operated power supply can be used with excellent results, hum and earthloop problems can be avoided much more easily with battery operation (2 12-V batteries will do if necessary).

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