Parietal cortex and brainstem were dissected and rapidly weighed on a Roller-Smith balance and subsequently homogenized in 1.0 ml of cold 0.3 *N* perchloric acid containing 1.0 m*M* EDTA. After centrifuging at 25,000 *g*/30 min, the supernatants were neutralized with 3.0 *N* $KHCO_3$.

The intermediates glucose, glucose-6-phosphate, lactate, pyruvate, ATP, ADP, and AMP were measured by the methods of LOWRY and PASSONEAU[10]. The Table compares the net levels of these intermediates in parietal cortex and brainstem of animals sacrificed by the two methods. Animals sacrificed by submersion in liquid nitrogen or in 2-methylbutane cooled to its freezing point with liquid nitrogen had similar metabolite values, and so these control data have been pooled.

Note that the lactate concentration was found to be significantly lower in both parietal cortex and brainstem of the chopped as compared to the submersed animals. Higher values as measured in the submersed group are associated with anoxia due to slow freezing of tissue[6]. The levels of the other metabolites measured were comparable in newborn rats sacrificed by the two methods.

In this study, we have developed and validated a new air-driven multi-bladed guillotine which rapidly sacrifices newborn rats, and fixes their brains such that regional studies are possible. We have compared commonly measured metabolites in animals sacrificed by the conventional submersion technique, and by the new chopping method. We find that only lactate, the most labile intermediate measured, was significantly lower in animals sacrificed by the chopping method. Since the small newborn rats have minimal insulative skin and bone covering their brains, submersed animals freeze almost as rapidly as the wafers. Major differences in the levels of all metabolites might be expected when comparing submersion vs. chopped adult animals. Modifications to the chopping apparatus which will allow accomodation of adult rats are currently being made.

The major advantage of this technique is that it facilitates regional studies in newborn rats. Thin, flat coronal wafers are readily dissected on dry ice into various anatomical regions. It is also sometimes advantageous to measure both enzymes and metabolites on the same sample. An advantage of the chopping method over sacrifice by microwave oven is that enzymes are not inactivated.

[10] O. H. LOWRY and J. V. PASSONNEAU, *A Flexible System of Enzymatic Analysis* (Academic Press, New York 1972).

Technical Note About Simultaneous Recording of Oxygen Partial Pressure and Neuronal Activity in Cat Cortex

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Summary. A method is described for simultaneous measurements of pO_2 and action potentials with microelectrodes using prefabricated gold wires. The construction and measuring with such gold electrodes is easier than with traditionally used platinum electrodes. Single electrode recording is done with a specially adapted electronic circuit, allowing the separation of both signals (pO_2 and action potentials. A measurement in cat brain illustrates this procedure.

In brain as in other tissues the local pO_2, when measured on a microscale, shows considerable spatial nonhomogeneities depending on the distance from the nearby capillaries, pO_2 in those capillaries, and O_2 consumption of the region under investigation. The following method allows a direct determination of pO_2 and action potentials (APs) simultaneously in the range of intercapillary dimensions. This requires a miniaturization of the measuring elements, which gives rise to certain problems described in detail.

A) *The gold microelectrode.* 1. *Construction.* The construction of the electrode, based on the principle published by ERDMANN[2], uses glass insulated gold wires, manufactured by the Battelle-Institute (Frankfurt/Main, Germany). After connecting of the gold wire to a copper wire by a conductive adhesive, it is threaded in a glass capillary so that it protrudes from the peripheral end of the capillary. To ensure mechanical stability, both wires are fixed at their respective ends of the glass with Araldit AY103 (Ciba-Geigy, Basel, Switzerland). The electrode tip is then abraded obliquely on diamond paste, so that the diameter of the tip is no more than 1–2 µm. This allows a good penetration of the electrode into the tissue, without gross damage to cellular structures. Finally, by dipping the tip in a 2% polystyrol-CCl_4 solution and fixing at 70 °C, the gold wire is coated with an O_2 permeable membrane. The loss of sensitivity which is often observed after the abrading process can be abolished by etching the gold on a length of about 20 µm in a KCN solution and new gilding of the tip in a gold chloride bath by applying 50 nA for 45 sec under microscopic control.

2. *Physical parameters.* Before use, the physical parameters of the electrodes have to be examined to control the quality of both wire and membrane. Examinations of polarographic curves confirm the finding of other authors[3] that the 'plateau' lies between 700 and 1200 mV. Therefore, we perform our pO_2 measurements at a voltage of (−1000 mV) against a silver-silver chloride reference electrode. At this voltage, the pO_2 dependent current curve has to be linear and the residual current at zero O_2 tension very low. It is recommended to apply a 1V potential for 24 h before use, thus permitting a suitable stabilization of the electrode. The current is only slightly temperature-dependent (0.03 nA/°C), while it is not affected by pH variations in the physiological

[1] II Hessen-Klinik, Erbach im Odenwald, German Federal Republic, BRD.

[2] W. ERDMANN and S. KUNKE, in *Advanced Experiments in Medicine and Biology* (Eds. D. F. BRULEY and H. I. BICHER; Plenum Press, New York 1973), vol. 37A, p. 261.

[3] H. METZGER, in *Oxygen Supply* (Ed. M. KESSLER; Urban & Schwarzenberg, München 1973), p. 164.

range. In addition, the response-time to a maximal change in pO_2 (from zero to air value) is short: on the average, 5–6 sec are necessary to reach 95% of the maximal value. The stirring -effect on the electrodes is tested by bubbling air through a NaCl solution. The variation of the O_2 current does not exceed 1% of the original value. Finally, the quality of the membrane coating is also tested by dipping the electrode in a protein solution, because the measuring current may be affected by such substances in the tissue. No variations are seen, proving the impermeability of the membrane.

3. *Calibration.* Polarographic O_2 measurement is, as is well known, affected by some error sources, due to physical characteristics of the electrodes as well as to problems specific to measuring in tissue. Therefore, the calibration must be very strict, in particular at zero O_2 tension. We found that, with the classic method of calibration in N_2-saturated NaCl, the pO_2 of such a solution is often much higher than zero, which led us to utilize a glucose-oxidase solution, consisting of a 20% glucose solution in which the available O_2 will be consumed by addition of glucose-oxidase (1,4 U/mg, Merck AG). With this method, one can prepare ampullas in which the pO_2 remains below 0.5 mm Hg over several weeks. The calibration is achieved by determining the maximal value in air-equilibrated NaCl.

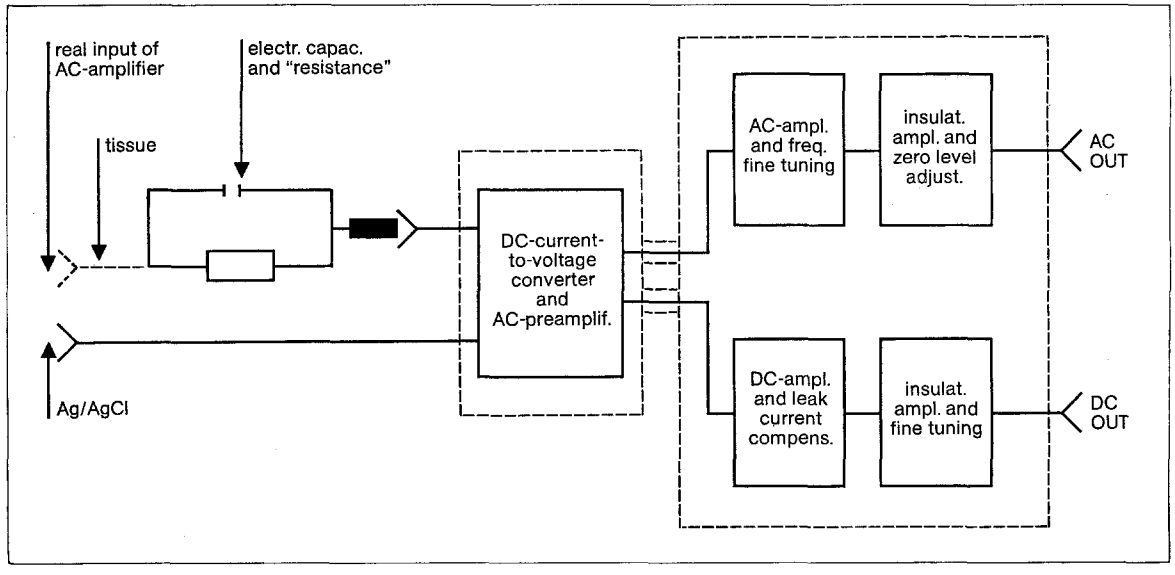

Fig. 1. Electronic circuit for simultaneous pO_2 and APs measurements.

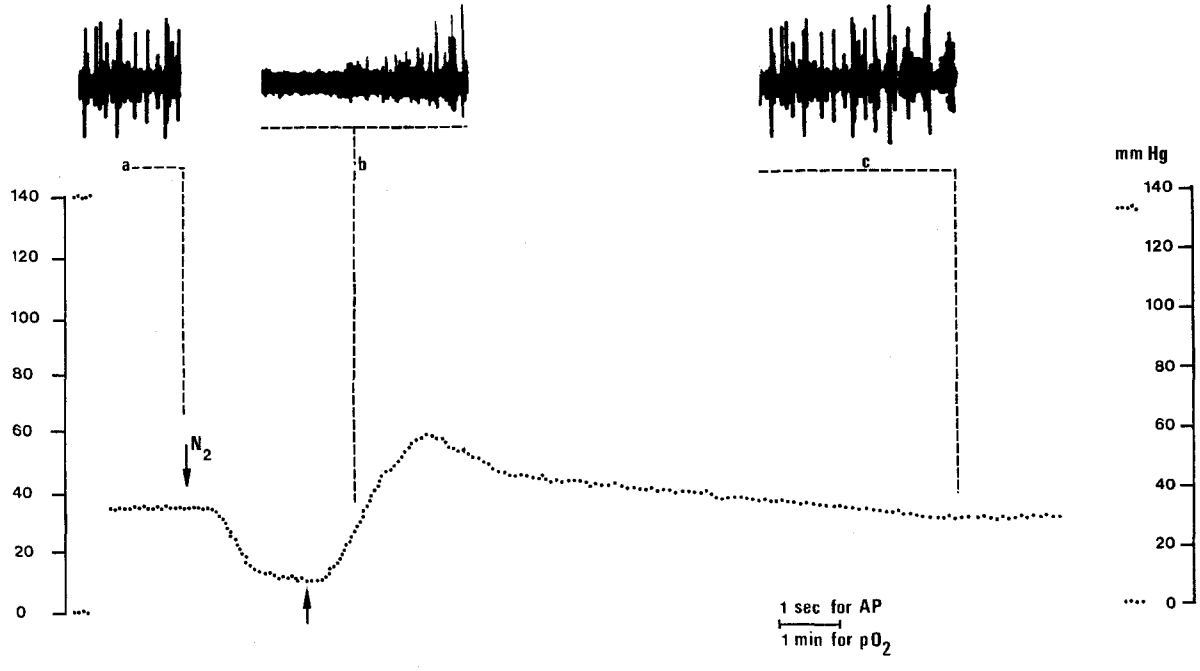

Fig. 2. Effect of hypoxia on pO_2 and APs in brain tissue. Note the calibration of the electrode before and after the experiment (along the pO_2 calibration scales).

B) *The recording system.* As shown above, recording of pO_2 and APs must be done at the same electrode tip. Both APs and pO_2 are low-level measurements which need high gain amplification and a good signal-to-noise ratio. If both measurements are done in one circuit, the input amplifier should be a perfect current amplifier with very low serial resistance and, at the same time, a good AC-amplifier with low noise amplification (Figure 1). The whole electronic system consists of 2 parts: one containing AC, DC amplifiers, potentiometers, meters, power supply and insulation amplifiers, and one other part containing the preamplifiers, which is installed inside the measuring cage, near to the electrode holder. Since the measured values may be affected by DC-potentials of the brain, the polarization voltage is achieved by a feedback-circuit which keeps the voltage constant between electrode and reference electrode, by comparing the actual value with a highly stabilized reference voltage. The difference between these 2 values may reach a maximum of 100 µV. This voltage-drop at the preamplifier input is negligible, so that the current amplifier has an ideal input resistance of almost zero. Using FET-input amplifiers, the whole current flows through the feedback-circuit and, by ohmic law, the output voltage of the DC current-to-voltage converter circuit is proportional to the current in the polarographic system and the ohmic resistance in the feedback circuit. The capacitance of the preamplifier input is extremely low (3pf), the input resistance very high (10^{12} Ohm). This enables 2 independent feedback circuits (for AC and DC) to be constructed in the preamplifier wiring. Therefore, the capacitance in the input circuit is low and static charges in the polarographic circuit are avoided. For recording of APs (= AC signal), the preamplifier circuit works as a low noise AC-amplifier. Hereby the capacitance at the electrode tip is included in the wiring diagram of the first FET-amplifier. The AC-amplifier needs the electrode capacity for stable working. For this reason, the real input of the AC-amplifying system is the tissue-side surface of the electrode membrane, and so the length between measuring point and AC-amplifier input is zero. In this way, APs are recorded with a good signal-to-noise ratio by adjusting the gain of the AC-amplifier. The following stages of amplifiers lead to a separation (by RC-coupling) into APs and pO_2 channels. In both channels, the zero level and the amplification can be adjusted. For monitoring of APs, an optoelectronic light emitter (for visual control), an oscilloscope and loudspeakers are used. All amplifiers are of course supplied by a highly stabilized voltage, while the reference voltage and the power supply for the preamplifier are separately stabilized. The whole system is supplied by 2 high power 18V accumulators, which are charged when the system is switched off.

C) *Experimental application.* In Figure 2a simultaneous recording of pO_2 and APs is shown, as obtained at a depth of approximately 500 µm in the parietal cortex of cat (body weight 2.7 kg, Nembutal 40 mg/kg). 20 sec after beginning of respiratory hypoxia (5% O_2, 95% N_2), the pO_2 drops and no sign of neuronal activity is seen after 20 sec. After 2 min hypoxia, return to air breathing is accompanied by a rapid increase of pO_2 which, following a period of overshoot, returns to the original value within a few minutes. The firing rate of the neurons rises progressively (Figure 2b) until it reaches the values before hypoxia, according to the findings of other authors[4,5].

[4] H. I. Bicher, D. F. Bruley, D. D. Reneau and M. H. Knisley, in *Bibliotheca Anatomica;* Karger Verlag, Basel 1973), vol. 11, p. 526.

[5] S. Kunke, W. Erdmann and H. Metzger, J. appl. Physiol. *32*, 436 (1972).

Formaldehyde-Schiff's Reagent as a Nucleolar Stain

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Summary. The use of formaldehyde-Schiff's reagent as a nucleolar stain has been described. Using different digestion procedures, it was confirmed that the stain is specific for RNA. It can be suitably used as a nucleolar stain, particularly in plant materials after a short TCA extraction, which probably extracts the nonbound RNA.

The use of formaldehyde-Schiff's reagent as a nucleolar stain was reported in a previous communication (Ghosh[1]). In plant materials this procedure has distinct advantages over other known methods. In this note the specificity of the stain is presented.

The stain is prepared by adding dilute formaldehyde in drops to freshly prepared Schiff's reagent (de Tomasi, cited in Pearse[2]) till the colour turns deep pink. The staining procedure has been described earlier (Ghosh[1]). After fixation, the material is treated with 5% TCA at 90 °C for 3 min, washed and stained in the formaldehyde-Schiff's reagent for 10–15 min, differentiated in 70% alcohol and squashed in a drop of 45% acetic acid.

The specificity of the stain was investigated in the Ehrlich mouse ascites tumour cells. Cell films were prepared and control preparations were stained as above. In some preparations the TCA extraction was omitted and the normal staining procedure was followed. Different extraction procedures were as follows: 1. Extracted in 10% perchloric acid at 4 °C for 20 h. Washed thoroughly in running water before staining. 2. Some preparations were treated with RNase (COI Worthington Biochemicals) in phosphate buffer (100γ/ml, pH 7.2) for 2 h and stained as usual. 3. RNase treated cells were extracted further as 1. 4. Some preparations were treated with DNase (free of RNase, Serva) in phosphate buffer (1 mg/ml, pH 7.2) containing 0.003 *M* $MgSO_4$ for 20 h at 37 °C. 5. Others were extracted with pronase (Calbiochem) in phosphate buffer (100γ/ml, pH 7.2) for 2 h. 6. Some preparations were treated with 5% TCA at 90 °C for 25 min to extract both DNA and RNA and stained as usual.

Figure 1 shows nucleolar staining in *A. cepa* roottip cells. Figure 2 represents cells in higher magnification showing nucleolar details. Staining of nucleoli can be seen

[1] S. Ghosh, Naturwissenschaften *61*, 687 (1974).

[2] A. G. E. Pearse, *Theorctidal and Applied Histochemistry*, 3rd edn. (Churchill, London 1968), vol. 1, p. 647.