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.

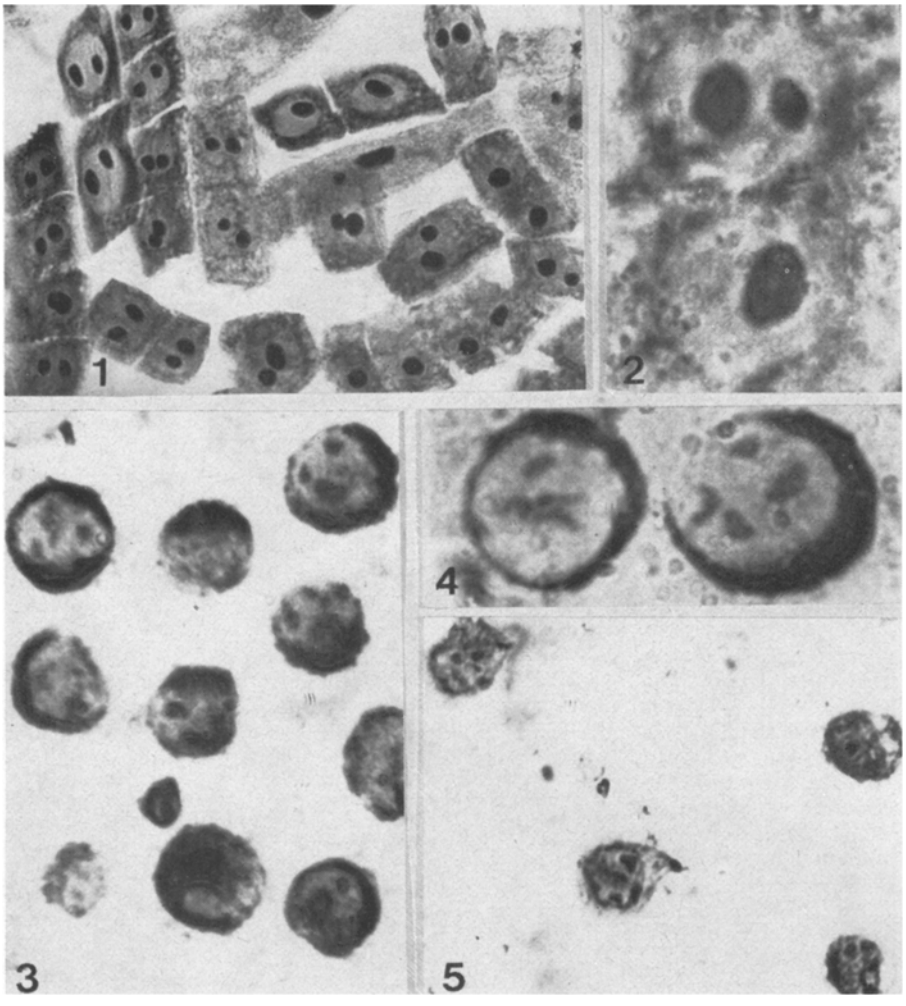
The stain is prepared by adding dilute formaldehyde in drops to freshly prepared Schiff's reagent (de Tomasi, cited in Pearse[2]) till the colour turns deep pink. The staining procedure has been described earlier (Ghosh[1]). After fixation, the material is treated with 5% TCA at 90 °C for 3 min, washed 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.



Figs. 1–5. Nucleolar staining through formaldehyde-Schiff's reagent. 1 and 2. Nucleoli in *A. cepa* roottip cells. 3. Mouse ascites tumour cells. 4 and 5. MAT cells treated with DNase and pronase respectively. 1, 3 and 5, ×600; 2 and 4, ×1,500.

in control preparations from ascites cells in Figure 3. When the TCA extraction was omitted, the cells revealed overall staining and prolonged TCA extraction resulted in complete loss of stainability. Preparations undergoing treatments 1 and 2 exhibited faint nucleolar and cytoplasmic staining, but no stain was obtained where RNase treatment was followed by perchloric acid extraction. Preparations from 4 and 5 exhibited only slight decrease in stainability (Figures 4 and 5). The results indicate clearly that the stain is specific for RNA. The short TCA extraction before staining is perhaps needed to extract the non-bound RNA and to obtain a specific nucleolar stain. The free amino groups of the pararosaniline molecules perhaps react with the nucleolar material.

Holding Plastic-Embedded Specimens for Sectioning in a Rotary Microtome[1]

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Summary. Three methods are evaluated for holding capsules of plastic-embedded tissue for rotary microtomy. Use of a V-block is rapid but deforms the capsule. Gluing the capsule to a supporting block is useful for reorientation of the capsule but otherwise time consuming. A metal adapter is easy to use, does not deform the tissue, and is the preferred method for routine microtomy.

Several laboratories use plastic to embed biological specimens which may be sectioned using a rotary microtome[2–12]. To produce quality sections, embedded tissue must be held securely in the microtome object clamp. This is especially true when sections of 0.5 to 5.0 μm of plastic-embedded tissue are cut. Since plastic-embedded tissues are usually cast in molds of gelatin or polyethylene capsules, the problem arises of how to hold the small cylinders of plastic-embedded tissue in the object clamp of a rotary microtome which was designed to hold rectangular blocks.

Rat liver was fixed in glutaraldehyde, embedded in glycol methacrylate and cast in size 00 gelatin capsules[6]. 3 methods for holding the plastic cylinders in an Ameri-