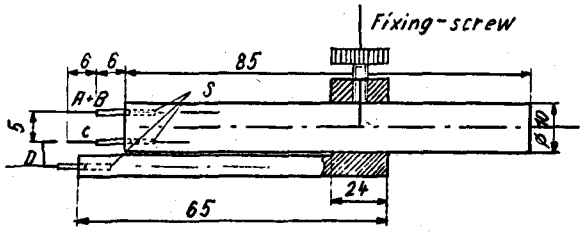

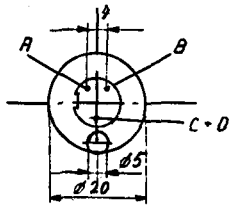

Fig. 2. Drawing of the stimulation electrode. All the dimensions are in mm. S, sockets; A, B, stimulation electrodes; B, C, signal detection electrodes.

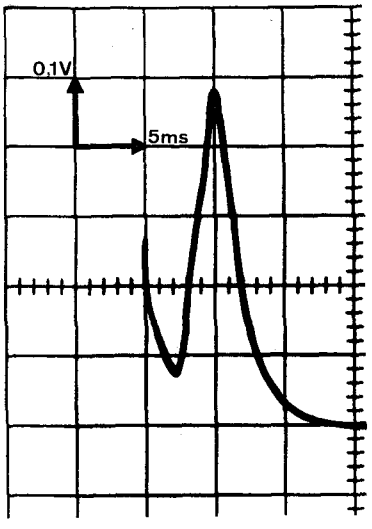

Fig. 3. Response of a piece of the electric organ stimulated directly in vitro (stimulation parameters: 100 V, 1.5 msec duration, 10 pps).

Principle. The sockets (S) of the bipolar stimulation electrodes and the signal detection electrodes are embedded with Araldite in a PVC tube and are localized on an area of 12 mm² (Figure 2). For the bipolar stimulation electrodes (A, B) and the 2 monopolar signal detection electrodes (C, D) non-insulated stainless steel electrodes with a diameter of 0.2 mm were used. The second signal detection electrode (D) connected with the mass of the organ piece can be changed with respect to its position. It has the great advantage of being able to stimulate organ pieces of different sizes in very small amounts of drug solution. All the electrodes are exchangeable by Amphenol plugs of the type 220–PO2–100. The response of a directly stimulated organ piece with this stimulation electrode is represented in Figure 3.

Zusammenfassung. Ein Elektrodensystem wird beschrieben, um kleinere und grössere Teile des Elektro-Organs von *Torpedo marmorata* direkt in vitro stimulieren und ableiten zu können.

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Preparation of Plant Material for Microautoradiography and Electron-Probe-Microanalysis: The Xylene Technique

Localization of ions in plant cells is usually based either on micro-autoradiography, on electron microscopy or on electron-probe-microanalysis (EPMA). In order to avoid any possible changes in the location of the examined ions during fixation and analysis, preparation must be made under such conditions as prevent their displacement.

The techniques used for preparation of plant material for electronprobe microanalysis and microautoradiography are usually freeze-drying[1–3] or freeze substitution[4,5]. Although freeze drying yields reasonable results in EPMA[6] and theoretically has an advantage over all other techniques, it is known to cause some tissue distortion[7,8], particularly at the subcellular level. On the other hand, freeze-substitution, which includes embedding of plant tissues in epoxy resin, suffers from uncertainties regarding the behaviour of ions during the dehydration procedure and is suspected of allowing some ionic movement[5,9,10]. In the present paper, a technique is described for preparation of high quality sections from frozen specimens which avoids both distortion of tissues and ion movement.

Segments of fresh leaves of *Panicum repens* L. and of *Cucurbita pepo* L. petiols were frozen and sectioned. Cross as well as longitudinal sections (8, 10, 15 20 and 40 µm thick) were cut in a cryostat at −20 °C. The frozen sections were placed on cold (−20 °C) aluminium plates precovered by chromalum glue ($CrK(SO_4)_2 \cdot 12H_2O$). Drops of cold (−20 °C) xylene were placed on top of the frozen sections. The sections were then left to dry overnight under the same conditions. The xylene drops evaporated after 2 h whereas it took a few hours before the ice sublimated. The material was deeply frozen throughout the procedure. The dried sections were then transferred to a dessicator at room temperature and left there for 1 h. Sections for EPMA were then coated with evaporated gold-paladium (60%/40%). Electron-probe X-ray microanalysis was conducted with a JXA-3A X-ray microanalyzer[6].

Displacement of soluble ions or their diffusion out of treated tissues during treatment with xylene was tested

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either by EPMA or by rinsing radioactive material in xylene and consequently analyzing the solution by liquid scintillation.

Sections treated with xylene were found to preserve the cellular details. Comparison between fresh and treated sections reveals that the various cell constituents, i.e. cell wall, cytoplasm, nucleus, chloroplasts and vacuole, remained in what seems to be their natural form. Thus, the quality of the sections obtained permitted the distinction of those sub-cellular constituents and localization of soluble ions in them without the need for elaborate, long and unreliable procedure.

Examination of the release of various ions from presoaked filter paper segments treated by the xylene technique was made. Paper segments were soaked in radioactive solution ($^{22}NaCl$ or $Na^{36}Cl$), dried and then either rinsed in water or xylene. Similar pieces of paper were deeply frozen after the first soak, and then rinsed in cold (−20 °C) xylene. The water and xylene were then analyzed for radioactivity by liquid scintillation. Ions were retained in the filter paper when subjected to xylene, but were leached when immersed in water. Examination by EPMA of the release of potassium ions from preloaded tissues which were treated by the xylene technique, proved the same.

It is thus suggested that the new technique permits the retention of ions at their original sites, eliminates the problems regarding movement of soluble ions during the dehydration procedure of freeze-substitution, and prevents the distortion usually caused by freeze-drying[11].

Summary. A new technique for preparation of plant material for electron probe microanalysis and microautoradiography is described. Frozen sections are treated with cold (−20 °C) xylene drops and frozen-dried at the same temperature. Such treatment preserves the cellular details of the sections, and avoids the distortions usually caused by fast freeze-drying. This procedure permits the precise localization of the elements within different subcellular compartments of plant material.

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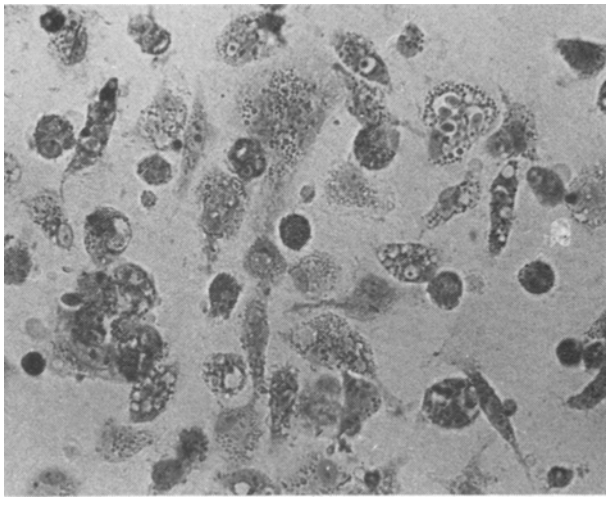
[11] The author's thanks are due to Prof. Y. Waisel and to Mr. B.Z. Weiss for their comments.

A Simple Method for Collection and Purification of Chicken Peritoneal Macrophages

It is usually difficult to obtain chicken peritoneal macrophages by the techniques commonly used[1,2]. In this short note we describe a new simple method for the recovery of these cells without using chemical or irritating agents.

Polystirene discs, 2.4 cm in diameter, were obtained from disposable petri dishes and implanted into the peritoneal cavity of 15-day-old chickens under light anesthesia. Chickens were sacrificed on day 4 after implatation and the discs were transferred to petri dishes, containing Eagle's minimum essential medium (MEM), care being taken to wash out blood clots. Discs appeared coated by a thin layer of cells consisting of 80% macrophages.



Chicken peritoneal macrophages after 20 h incubation in MEM + fetal calf serum 8%. ×250.

The cell layer was torn out from the substrate and treated with trypsin 0.25% in Puck's saline solution, pH 7.2, for 10 to 15 min and the resulting cell suspension centrifuged at 800 × *g* for 5 min. Cell viability was controlled by the exclusion dye test[3] and consistently found to be more than 96%.

Cells were suspended at 4×10^5 cells/ml, in MEM additioned with 8% fetal calf serum, streptomycin, 100 µg/ml, Penicillin, 100 IU/ml. Cells were seeded in glass Demeter flasks (14 ml/flask) and incubated in an atmosphere of air and CO_2 (5%) at 37 °C.

After a 20 h incubation, the culture presented round and spindle large cells (Figure). At this time the medium was discarded, cells were washed twice with growth medium and detached from the flask with a rubber policeman.

We classified these large cells as macrophages because of their morphology, adherence to glass and capacity to phagocytize indian ink particles. In this way we obtain 3 to 4 millions purified macrophages per chicken.

Summary. The authors describe a simple method for collection and culture of chicken peritoneal macrophages. The macrophages are collected from peritoneum with polystirol disks on the surface of which they are subsequently cultured in vitro.

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