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.

A. Ramati and Nili Liphschitz

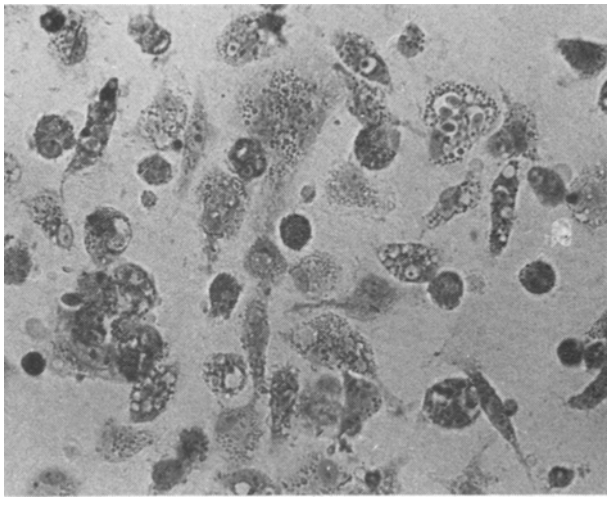
Department of Botany and Institute of Archaeology, Tel Aviv University, Tel Aviv (Israel), 24 February 1975.

[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.

S. Micalizio and C. della Bruna

Farmitalia, Gruppo Montedison, Viale E. Bezzi 24, I-20146 Milano (Italy), 17 March 1975.

[1] S. R. S. Rangan, Expl. Cell Res. *46*, 477 (1967).
[2] M. E. Rose, Infect. Immunity *10*, 862 (1974).
[3] C. P. Engelfriet and A. Britten, Vox Sang. *10*, 660 (1965).