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NOTES ON TECHNIC

A Simple Procedure for Autoradiography of Keratinocyte Cultures

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Although cell cultures usually grow as a monolayer, there are some circumstances in which they become multilayered or stratified. This is the case with epidermal cell cultures where skin keratinocytes grow attached to the bottom of the culture flask, but as they differentiate lose contact with the bottom and pile up on top of

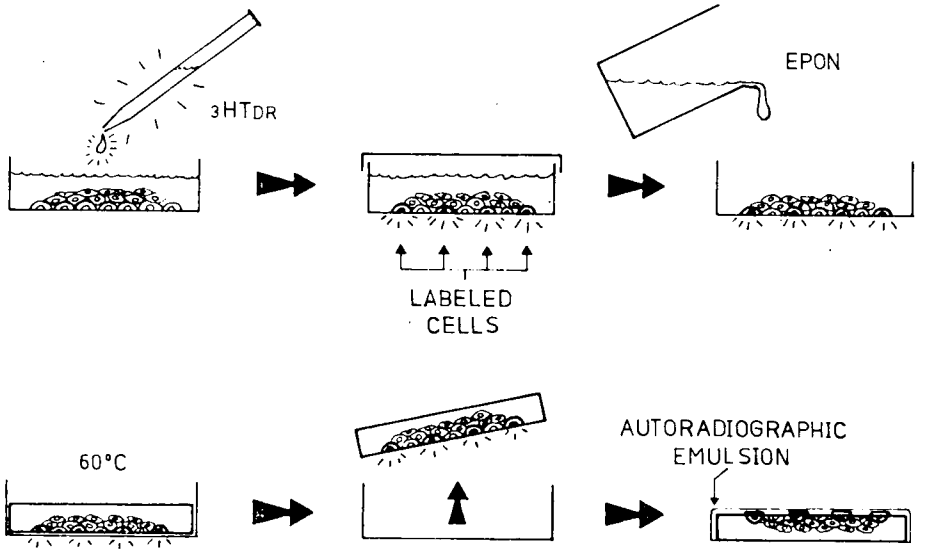

FIG. 1. Diagram illustrating the autoradiographic procedure for keratinocyte culture.

the cell colony to create a situation resembling the structure of skin *in vivo* (Rheinwald 1980). In this way, proliferative cell pools are attached to the bottom of the culture flask and are partly covered by nonproliferative, differentiated cells. This particular arrangement makes cell kinetic studies difficult because such studies are usually carried out by autoradiographic evaluation of the uptake of tritium-labeled DNA precursors (mainly thymidine) into the nucleus of cells synthesizing DNA. As tritium beta particles are very weak, they are not able to reach the autoradiographic emulsion because they are blocked by the differentiated layer of cells. This difficulty has been partly overcome by using ^{14}C precursors of DNA (Steinberg and Defendi 1979).

An alternative procedure which allows the autoradiographic study of the cells at the bottom of multilayered cultures is described here (Fig. 1). This technique was performed in keratinocyte cultures cultivated according to the technique of Rhein-

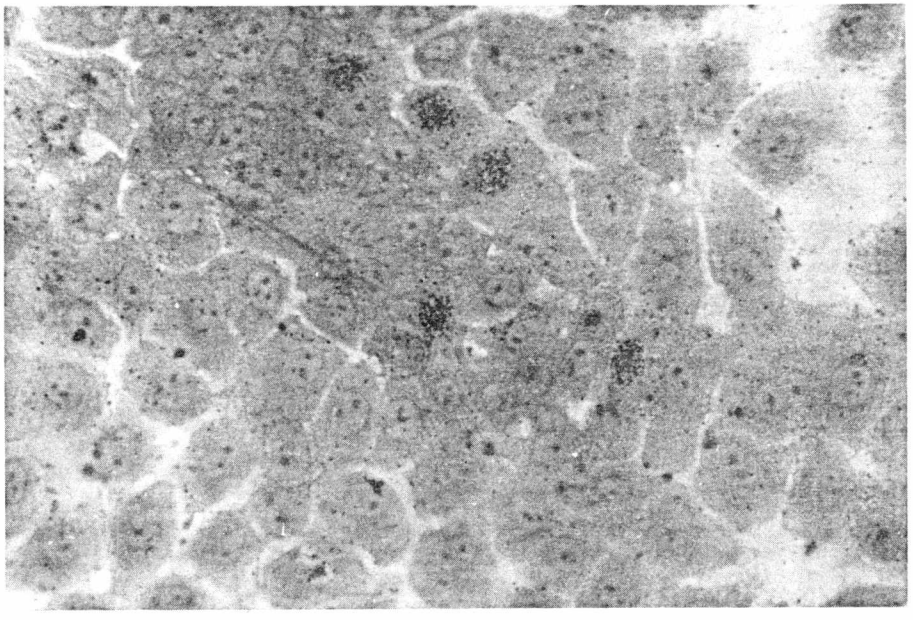

FIG. 2. Autoradiograph of a keratinocyte culture. 270 ×.

wald and Green (1975). After 24 hours of incubation in 2 μCi/ml tritiated thymidine, keratinocyte cultures grown in 30 mm plastic dishes (Falcon) were fixed in absolute ethanol and embedded in a layer of Epon (Luft 1961) approximately 1 mm thick. Once the Epon is cured (24 hours at 60 C) the plastic blocks can be easily detached from the tissue culture plate in the same way as for electron microscopy of monolayer cultures (Tsuruhara *et al.* 1971). The cells at the bottom of the culture, which is included in the plastic block, are found immediately below the surface of the detached side of the block. Labeling of these cells was revealed by applying Kodak NTB 2 autoradiographic emulsion to that side of the plastic block. After 48 hours of exposure the autoradiographs were developed in D-19 (Kodak). Classic alkaline basic dyes usually used to stain Epon thick sections failed to adequately stain these autoradiographs. Preheating the blocks for 3 minutes in 0.1 NaOH before dipping them into the emulsion greatly improved toluidine blue (1%, pH 9) staining of developed autoradiographs (Fig. 2).

This technique can be applied to other culture situations in which labeled cells are partly or totally masked by other cells. It can also be used in those cases in which cells have been cultivated on glass surfaces that do not allow good microscopic observation.

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Acrylic Emulsion Method For Adhering Glycolmethacrylate Tooth Bud Sections to Glass Slides in Caustic Solutions and Strong Solvents

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Detachment of sections from glass slides is a problem in histochemical procedures which require the use of caustic solutions such as hot alkalis and also strong solvents.

Conventional adhesives, *e.g.*, chrome gel, albumin, B.S.A., silicone, and epoxy, failed to keep JB-4 glycolmethacrylate (Polysciences, Inc., Warrington, PA) plastic embedded tooth bud sections attached to glass slides in a test for protein-bound sulfhydryl groups (Barrnett and Seligman 1952). The hot (50 C) alkaline solution of 2,2′-dihydroxy-6,6′-dinaphthyl disulfide (DDD), pH 8.5, and subsequent alcohol and ether washes detached the sections.

Loss of time and aggravation were experienced when sections were processed individually and mounted on glass slides at the end of the procedure. Since it was also desired to perform immunoperoxidase or immunofluorescent procedures where detachment of sections was a problem but where covering the sections was contraindicated, dye-permeable overlayered adhesives were not considered.

Glycolmethacrylate sections are probably attached to the slide primarily by mechanical adhesive joints rather than by a chemical bond (Lee 1974). The sequence of (1) swelling of glycolmethacrylate sections and (2) wetting of the slide by caustic solutions, especially by strong alkali solutions such as the DDD, first breaks the adhesive joints and then washes the sections off the slide. The hygroscopic nature of the glycolmethacrylate plastic probably causes swelling of the section. The strong alkalis and solvents act as wetting agents.

An acrylic resin emulsion method of smearing a glass-specific adhesive over two locations, 1) perforations in the plastic surrounding the tissue section and 2) the glass slide for several millimeters around the plastic, was developed to resolve the problem.

Material and Method. The adhesive Rhoplex N-619 acrylic resin emulsion liquid (Rohm and Haas Company, Philadelphia, PA*), a milky aqueous acrylic resin liquid with a mild ammoniacal odor, has a specific adhesion to glass. It contains acrylic polymer combined with ammonia and some residual monomers.

* Available in research quantities through Polysciences, Inc., Warrington, PA, as Polysciences' Tissue Adhesive II-10-D.