Relative quality of methods used to hold cylinders of plastic-embedded tissue during microtomy on a rotary microtome

Criteria	Methods evaluated		
	V-Block	Cement on carrier	Metal adapter
Lack of tissue distortion	+	+++	+++
Ease of use	+++	+	+++
Speed of use	+++	+	+++
Uniformity of section thickness	+	+++	+++

can Optical-Spencer 820 rotary microtome were evaluated for ease of use and quality of sections produced. 2 µm sections were cut with steel knives (Table).

The simplest method for holding the cylinders for sectioning was to use the V-block assembly included with the microtome as part of the object clamp assembly. The cylinders were placed in the V-groove and were clamped securely. Occasionally the 3-point clamp caused cylinders of the more brittle plastic to fracture. On the other hand, with cylinders of softer plastic, tight clamping distorted the cylinders causing undesirable artifact in the tissue. The most common complaint was that with time the cylinders tended to loosen in the V-block assembly resulting in the sections of non-uniform thickness.

The second method was to cut the cylinder 5 mm below the tissue and cement this piece of plastic to a plastic or metal object carrier of suitable size to fit the microtome. This method is the choice of several laboratories[4, 8, 12–15]. This method was time consuming but was otherwise satisfactory.

The third method was to use a metal adapter originally described by BECKEL and HABOWSKY[3]. For size 00 capsules an 8 mm diameter hole was bored through the center of a 16 mm cube of brass. The cube was then cut longitudinally through the bored hole. The 2 halves of the adapter fitted snugly around the capsules and provided parallel surfaces for the microtome object clamp. For capsules of different sizes, the diameter of the hole bored in the brass cube must be suitably matched.

The use of the metal adapter is preferred over the V-block assembly because it allows the object clamp of the microtome to be tightened beyond the point where plastic cylinders would fracture or become distorted if held in the V-block. In addition, using the adapter is faster than cutting and cementing a portion of the plastic cylinder on an object carrier. Plastic cylinders can be changed in the adapter in less than 1 min. The cutting and cementing procedure takes ten minutes or longer per cylinder and is used in our laboratory only when we wish to reorient the tissue for sectioning.

[1] Supported in part by the U.S. Veterans Administration Hospital, Hines, Illinois.

[2] C. A. ASHLEY and N. FEDER, Arch. Path. *81*, 391 (1966).
[3] W. E. BECKEL and J. E. J. HABOWSKY, Stain Techn. *35*, 343 (1960).
[4] W. C. DE BRUIJN and S. J. MCGEE-RUSSELL, J. R. microsc. Soc. *85*, 77 (1966).
[5] W. J. CATHEY, Stain Techn. *38*, 213 (1963).
[6] M. B. COLE, JR. and S. M. SYKES, Stain Techn. *49*, 387 (1974).
[7] D. L. DE GIUSTI and I. EZMAN, Trans. Am. microsc. Soc. *74*, 197 (1955).
[8] N. FEDER and T. B. O'BRIEN, Am. J. Bot. *55*, 123 (1968).
[9] G. D. KUHN and E. L. LUTZ, SR., Stain. Techn. *33*, 1 (1958).
[10] C. L. RUDDELL, Stain Techn. *42*, 119 (1967).
[11] C. L. RUDDELL, Stain Techn. *42*, 253 (1967).
[12] C. L. RUDDELL, Stain Techn. *46*, 77 (1971).
[13] G. L. HUMASON, *Animal Tissue Techniques*, 3rd edn. (Freeman, San Francisco, Calif. 1972).
[14] C. J. ROHDE, JR., Stain Techn. *40*, 43 (1965).
[15] B. SIMS, J. Microsc. *101*, 223 (1974).

A New Technique for Dissociation of Hair Follicles into Single Cells

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Summary. A method is described for the complete dissociation of mouse hair follicles into a suspension of single cells suitable for cell culture. Dermal tissue containing hair follicles is digested sequentially with trypsin and chondroitinase ABC under mild conditions.

Cell culture techniques are widely used to study the behaviour and properties of cells from a variety of embryonic and adult tissues. To obtain a suspension of cells suitable for culture, the tissue is generally chopped finely and stirred for some time in dilute proteolytic enzyme solutions, often in the presence of chelating agents. Although trypsin is the enzyme most often used, many others have been employed to help dissociation in more difficult cases – particulary for adult tissues. Supplementary enzymes used have included collagenase, elastase, papain, hyaluronidase, pancreatin and pronase.

In developing a method for studying the behaviour of hair follicle cells in a culture system, some success in obtaining single cell suspensions from young mouse skin has been achieved[1, 2]. In this method, the dermal layer containing lower follicle bulbs was dissected from the skin and incubated with stirring in the presence of trypsin solution containing EGTA (EGTA = ethylene glycol *bis*(β-aminoethyl ether)-N–N′-tetra acetic acid (Calbiochem)). Although viable keratin producing cells were obtained[2], it was apparent by microscopic examination of the residual tissue that the inner, germinal regions of the follicle bulbs were still largely intact. Further digestion of undissociated bulbs with solutions of the above mentioned enzymes failed to free more cells into the solution. When attempts were made to dissect these bulbs with needles, the cells remained fixed to sticky strands of material contained within the inner parts of the bulb.

Much of this sticky material is probably acid mucopolysaccharide, as hair follicles are known to contain large amounts of the substance[3], and it would not be degraded by any of the enzymes so far used. This paper describes a new technique which enables complete dissociation of hair bulbs into single cells.

[1] R. FRATER, J. invest. Derm. *64*, 235 (1975).
[2] R. FRATER, in preparation.

Number of cells released from skin by enzymic digestion

Experiment	Cells released per 10 mg tissue (total)	Non-viable cells per 10 mg tissue	Viable cells (%)
Trypsin + EGTA + chondroitinase	140,000	43,500	69
Trypsin + EGTA (control)	31,000	16,700	45

Materials and methods. Albino mice (5–6 days old) were killed by decapitation and a small piece of skin removed from the mid-dorsal area. Pieces of dermis containing the lower hair follicle bulbs (approx. 0.5 × 0.5 × 1 mm) were dissected from the under side of the skin, blotted, weighed, and transferred to 1 ml aliquots of trypsin solution containing 0.3% trypsin (Difco I:250) and 0.05% EGTA in sterile Hanks' solution (calcium- and magnesium-free). Incubation was carried out for 10 min at 37 °C in sealed tubes with gentle shaking every 2 min. Pieces of this tissue were then incubated for a further 10 min in 1 ml of the trypsin solution (control) or, after washing in Hanks' solution, transferred to 1 ml of Hanks' containing 5 units of chondroitinase ABC (Sigma) and incubated for 10 min at 37 °C with gentle shaking. After this, the contents of each tube were sucked up and down for 30 sec with a Pasteur pipette, and the resulting cell suspensions diluted with an equal volume of 0.1% nigrosine in Hanks' solution. This dye stains the cytoplasm of damaged cells, but is not taken up by viable cells[4]. After thorough mixing, a drop of each suspension was placed in the chamber of a haemocytometer and the cells (viable and non-viable) counted.

Results and discussion. The results (see Table) show that nearly 5 times as many cells were released by trypsin plus chondroitinase as compared to trypsin alone, and also that the percentage of viable cells was much higher in the first case (69% as compared to 45%). Microscopic examination of the residual tissue when the new method was used showed only long strands of collagen and parts of lower hair shafts – no intact follicle bulbs were seen. In contrast, the control digest contained in the residual tissue a great number of recognizable follicle bulbs. Some fresh pieces of dermis were also incubated with chondroitinase alone (5 units per ml of Hanks' solution) and, after 30 min at 37 °C it was apparent that some dissociation of follicle bulbs had occurred, but not to the degree found using trypsin plus chondroitinase. The use of the 2 enzymes sequentially thus offers a means of obtaining single viable cells from hair follicles representative of the whole hair bulb.

[3] B. SYLVÉN, Expl. Cell Res. *1*, 582 (1950).
[4] J. P. KALTENBACH, M. H. KALTENBACH and W. B. LYONS, Expl Cell Res. *15*, 112 (1958).

CONGRESSUS

France
29th International Meeting on Electrical Phenomena at Membrane Level

in Saclay, 12–15 October 1976

The main topics are: 1. Bioenergetical study of coupling mechanisms. 2. Electrical phenomena at exitable membrane level. The scientific program and registration information will be available by: Dr. C. Troyanowsky, General Secretary, Société de Chimie physique, 10, rue Vauquelin, F–75231 Paris Cedex 05, France.

Federal Republic of Germany
First International Congress for Research on Medicinal Plants

in München, 6–10 September 1976

Organized under the auspices of Gesellschaft für Arzneipflanzenforschung, the Phytochemical Society of England and the International Association for Plant Tissue Culture the Congress will be devoted to the following topics: A) New natural products and plant drugs with pharmacological or therapeutical activity. B) Plant tissue culture and its bio-technological application. Further information for section A: Dr. P. Wolff or Prof. H. Wagner, Institut für Pharmazeutische Arzneimittellehre, Karlstrasse 29, D–8000 München 2, Federal Republic of Germany. For Section B: Prof. E. Reinhard, Pharmazeutisches Institut, Auf der Morgenstelle 8, D–74 Tübingen, Federal Republic of Germany.

Canada
Third International Symposium on Pharmacology of Thermoregulation

in Banff, 14–17 September 1976

The Symposium will be held at the Banff Centre and further details about registration may be obtained by the organizers: Prof. K. E. Cooper, Division of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4; or by Prof. P. Lomax, Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024, USA; or by Prof. E. Schönbaum, Peelkensweg 4, 4274 Venhorst N. Br., The Netherlands.

CORRIGENDUM

MARIANNE E. SCHWAGER-HÜBNER and M. C. GNÄDINGER: *Synthesis of Sulfated Glycosaminoglycans by Three Cell Types of the Rabbit Cornea in Culture*, Experientia *32*, 15 (1976). On page 16 in the first paragraph 2 lines have been omitted. The two first sentences should read as follows:

After 21 days of incubation, 50 μCi $Na_2{}^{35}SO_4$ (NEN, specific activity 859 mCi/m*M* were added to the medium of each culture for 4 days. At the end of the exposure to the precursor, the medium was withdrawn and the cells homogenized.