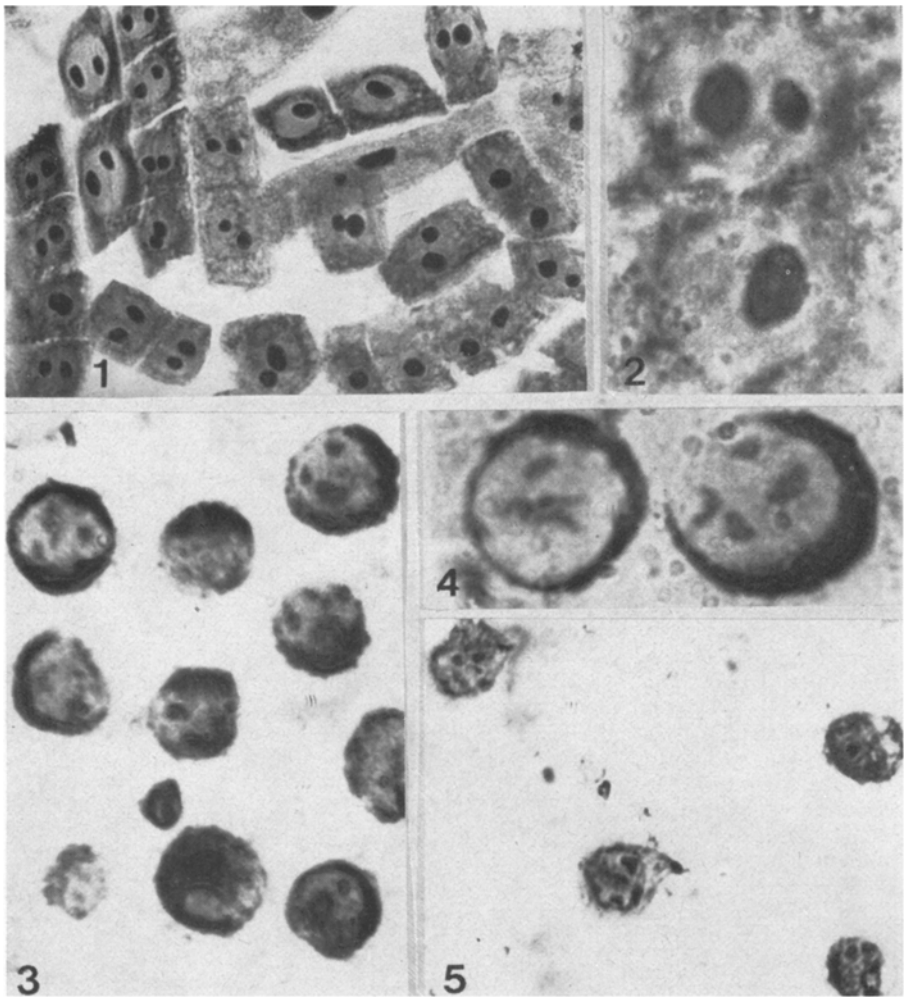

Figs. 1–5. Nucleolar staining through formaldehyde-Schiff's reagent. 1 and 2. Nucleoli in *A. cepa* roottip cells. 3. Mouse ascites tumour cells. 4 and 5. MAT cells treated with DNase and pronase respectively. 1, 3 and 5, ×600; 2 and 4, ×1,500.

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-

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.

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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.

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