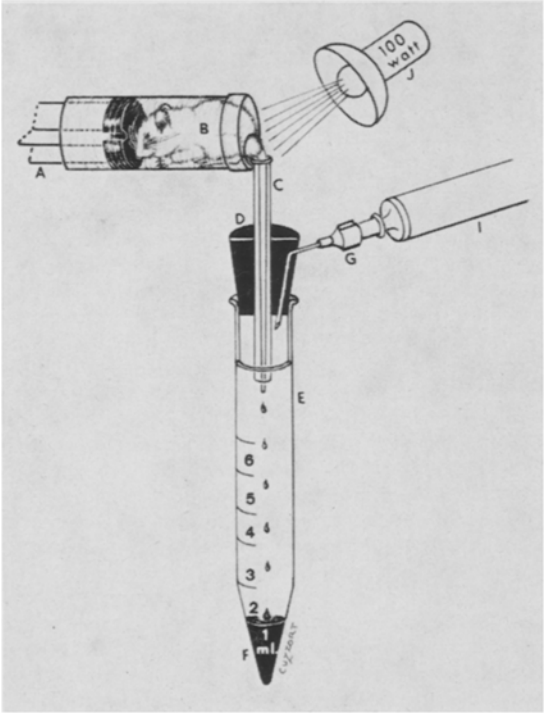
practical in some cases to immobilize the mouse by taping its feet and hands to a piece of cork). Then the tail must be washed with surgical soap, rinsed and dried Next the tip of the tail is cut with a sterile scalpel and then introduced into the sterile tube. The tube, the stopper and the graduated test tube are assembled as shown in the Figure.

A needle connected to a vacuum pump is then introduced into the test tube through the rubber stopper so that the suction pulling down the tail will effect a complete closure of the tube. The vacuum thus created will prevent air from passing into the test tube and enable the blood to be sucked into it.



A) Plunger; B) plastic container; C) sterile plastic tube; D) rubber stopper; E) graduated sterile test tube; G) 20 Gauge needle; I) vacuum; J) 100 Watts lamp.

If bleeding is slow, it is helpful to apply the heat of a 100 watts lamp to the mouse from a distance of a few cm for 2–3 min, producing vasodilatation. In this way, one can usually draw from 0.75 to 1 ml of blood from each mouse weighing approximately 26–32 g. If the animal appears weak after bleeding, it is useful to administer 2 ml of 5% dextrose in saline i.p.

Blood cultures. Blood, obtained with the method described, is transferred immediately to a tube of thioglycollate medium, incubated at 37 °C, and examined throughout 2 weeks.

Results. The method described has been used many times over a period of 2 years without accurate records being kept. For the purpose of having an exact report, it was used on 28 C3H/He mice obtained from the Jackson Laboratories, Bar Harbor, Main. The results are summarized in the Table.

We conclude that: 1. 1 ml of blood can safely be taken from healthy mice, weighing more than 26 g but not more than this (for example 1 mouse weighing 28 g, died after 1.30 ml of blood was obtained from it. another weighing 27 g died after 1.25 ml). 2. With mice weighing less than 26 g the safe limit is 0.55 ± 0.25 ml. 3. Blood can be collected by cutting about 1 mm of the tip of the tail at intervals of 30 days. 4. The technique results in generally 'clean' blood samples, and in the majority of occasions there are bacteriologically sterile.

Riassunto. Viene descritto un semplice metodo che permette il prelievo di sangue dai topi senza che questi vengano sacrificati. Il metodo è raccomandato in tutti quei casi in cui si richiede il minimo stress da parte dell'animale ed in quei casi in cui, come in immunologia, si vogliono ottenere campioni di sangue dallo stesso animale in varie riprese.

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Synthetic Coating: An Improvement in Ultracryotomy

The principal advantages of using semi-thin and ultra-thin frozen sections, instead of sections made of embedded material, have been described repeatedly[1–7]. With the development of commercial equipment for ultracryotomy (Sorvall MT-2B, FTS LTG2; LKB CryoKit; Reichert OMU3, FC2) and the description of several methodical variants[8–13], the interest in this procedure has continued to grow. Since not all technical difficulties involved in ultracryotomy have been overcome, however, we attempted to develop a procedure which not only allows better cutting of the frozen tissue but also facilitates manipulation of even ultra-thin sections and enables determination of section thickness based on the occurrence of interference colours.

Method. By immersion in liquid nitrogen unfixed kidney and liver tissue was frozen onto a silver (Reichert) or else onto a copper slide (Sorvall) and then attached to

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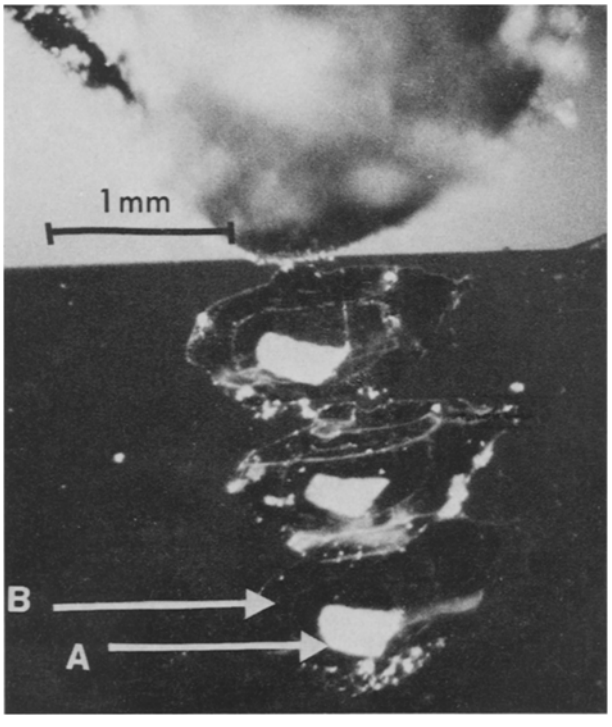
the specimen holder of the cooling units which had already been cooled to working temperature. Following this the object was trimmed under low magnification to a size of approximately 0.2–0.3 mm. In order to coat the object with a fast adhering synthetic sheath, either polyvinylacetate (PVA) or polyvinylformaldehyde (PVF) in a 5–20% solution of methylene chloride or ethyl chloride, precooled to the working temperature of −50 to −90 °C, was used. These solutions were frequently coloured with Sudan black B for better observation and demonstrability. To prevent the solvent from penetrating unnecessarily deeply into the tissue, a small amount of the above-mentioned solutions can first be brushed onto the lateral surface of the block, whereupon the solvent evaporates within less than 1 min, forming a first adhering synthetic layer. According to the purpose of the experiment, this synthetic coating can be gradually reinforced up to a thickness of 0.5 mm and more (see Figure). At very low temperatures, it is advisable to use ethyl chloride (melting point: −140 °C); at temperatures of −50 to −90 °C methylene chloride (melting point: −96.7 °C) can be used. Experiments have shown that, using the proper procedure, the synthetic coating is completed within 5 min.

When completely dry the object (usually at −70 °C) was cut semi-thin or ultra-thin with a glass knife (knife angle: 45°, free angle: 6°). During some experiments DMSO-H_2O 60:40 (V/V) served as a floating medium.

Analogous to a previously described procedure[14] for cutting frozen sections of whole animals, in some experiments a 1% solution of PVA or PVF in methylene chloride or ethyl chloride was used in addition to the above-mentioned lateral coating to reinforce each individual section at the cutting surface.

Results and discussion. The synthetic coating described here allows for the production of technically good semi-thin and ultra-thin frozen sections of unfixed tissue at working temperatures under −70 °C (Figure). Advantages of this procedure especially in ultracryotomy are: 1. Improved cutting of the frozen object surrounded by the synthetic coat and therefore considerably less deformation, folding, and tearing of the sections; 2. Better manipulation especially of sections of small, fast-freezing objects by hair, brush, or pointed tweezers or else easy removal from the floating medium; 3. Determination of section thickness with the aid of interference colours in the surrounding uncoloured synthetic coat, as is otherwise done with plastic-embedded tissue; if the synthetic solution contains Sudan black B, the colour intensity can be used as an indicator for section thickness; 4. Contrary to 'gelatin embedding'[1,2,7,11], the synthetic coating method can be carried out at low temperatures with corresponding pre-cooled solutions; soaking the tissue in embedding medium is not necessary.

Possible disadvantages of the synthetic coating are: 1. The slightly greater amount of time required initially in preparing the synthetic coat; in comparison to the duration of the whole experiment, however, this can, in our opinion, be ignored; 2. Differences in the hardness of synthetic material and frozen tissue can impair the quality of the sections; however, this disadvantage can be minimized by optimal proportioning of the synthetic coat and probably be eliminated completely by an appropriate choice of synthetic material; 3. A certain, though only superficial, penetration of the solvent into the tissue, undesirable in some respects, is probably necessary, on the other hand, for the firm adhesion of the synthetic material to the object. In view of the minimal solubility of our solvents in water, e.g. methylene chloride only 1:40, they could reach only the lipid phases of the frozen tissue block even when applied quite heavily; artificial distortions of water phases are probably not to be expected at all. If the experiment requires that alterations or dislocations of lipidsoluble tissue components also be excluded, then the contact between solvent and tissue should, and can be, reduced by gradual application of the synthetic coat, and thereby limited to a superficial tissue zone to be specified by control experiments.



Example of 3 sections from a block of frozen kidney tissue with synthetic coating; section thickness 200 nm. A) frozen section; B) synthetic coat in situ on the glass knife.

Zusammenfassung. Es wird eine methodische Verbesserung der Ultrakryomikrotomie unfixierten Gewebes beschrieben. Das Prinzip dieser «Kunststoffumhüllung» besteht darin, den Gewebeblock durch Auftragen einer Kunststofflösung – verwendet wurden auch in der Kälte noch ausreichend flüchtige organische Lösungsmittel – mit einem Kunststoffmantel zu umgeben. Dadurch wird die Schneidbarkeit des gefrorenen Gewebes verbessert, die Manipulierbarkeit auch von ultradünnen Gefrierschnitten erleichtert und eine Dickenbestimmung der Schnitte anhand von Interferenzfarben ermöglicht.

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