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A TECHNICAL NOTE FOR IMPROVEMENT OF THE E12 TECHNIQUE J.

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INTRODUCTION

The E12 plastination technique is used for impregnation of tissue slices with transparent epoxy resin (1). To obtain the most useful specimens, one must start with sections no thicker than 2-3 millimeters. Several methods have been suggested for the production of acceptable sections. These include the conventional sawing of specimens frozen at -20°C (2), the sawing of frozen specimens with additional ice laid against the stop (3) and the sawing of frozen specimens using specially constructed stops in which cooling is accomplished with a counter-current device (4).

The first two of these methods are not suitable for producing slices with a large surface area such as whole-body transverse sections. Neither are they satisfactory for producing multiple serial sections that must be cut in one session. The third may be used with large specimens and in serial sectioning but breakdown or other operational faults are likely to occur. The purpose of the present investigation was to develop a reliable and simple means of producing tissue sections appropriate for use in the E12 technique.

MATERIALS AND METHODS

A sectioning apparatus was developed by altering a conventional band-saw (Berkel 444, manufactured by Maschinenfabrik K.M. Reich, Niirtingen, Federal Republic of Germany). Modification of the sawing table and the stop were required.

SAWING TABLE: The table supplied with the saw was removed and replaced with one constructed from a plate of 4mm sheet aluminum. The replacement table measured 600 x 360 mm. Backward and forward motion was provided by four wheels running on appropriately aligned rails. Special care was taken to obtain a smooth, gliding action with the table running exactly parallel to the stop.

THE STOP: The stop was cut from 20 mm sheet aluminum and measures 800 x 200 mm. An oblong aluminum container (840 x 150 x 35 mm with a wall thickness of 2 mm) served as a receptacle for liquid nitrogen. Its seams were sealed with a durable, highly elastic, leak-proof glue. A 14 mm hole was bored at each end of the upper surface, one to allow the nitrogen to be introduced and the other acting as a vent. This container was fastened to the back of the stop with screws. The clamping mechanism which holds the stop in place was arranged in such a way as to allow expansion and contraction due to temperature change.

RESULTS AND PROCEDURE FOR USE

A picture of the modified saw is shown in Figure 1. A view of part of the stop is shown in Figure 2. Corresponding construction diagrams are provided in Figure 3.

The procedure recommended for using this saw is as follows:

1. The specimen from which slices are to be cut is frozen thoroughly at -70°C. The time required for this will, of course, vary with its size. A torso, for example, should be kept at -70° for one week.

2. The saw is fitted with an appropriate blade (e.g., a blade of type B or G from Fischmeister, Pansdorf, Federal Republic of Germany).

3. A millimeter scale, calipers and a small brush are placed close at hand. These will be used in setting the stop, monitoring the thickness of the slices and cleaning the slices.

4. Two acetone baths, prepared in advance, are made available. This acetone must have been kept in a freezing cabinet at -25°C for at least two days before the saw is used.

5. The stop (including funnel and supports) is cooled to -70°C for two hours before the saw is used.

6. Liquid nitrogen (20 liters) is made available.

The saw is ready for use when the stop has been aligned 2 mm from the blade and the container filled with liquid nitrogen (approx. 3 liters). The specimen is then cut into 2 mm slices. If there is any sign of thawing, more liquid nitrogen should be added.

After quickly checking its thickness with the calipers, each slice is transfered immediately to an intermediate -25°C acetone bath where it is carefully freed of ice fragments and sawdust with the brush. The cleaned sections are then stacked in a second acetone bath at -25°C, where dehydration can begin.

DISCUSSION

The difficulties involved in preparing sections to be plastinated with the El2 technique arise from the fact that heat generated by the friction of the saw blade tends to soften the tissue. And it is impossible to obtain clean, smooth sections unless the tissue being sawed is kept frozen. The only way to eliminate this difficulty is to employ some means of maintaining the specimen at a temperature below freezing while it is being sawed. The method described here is based on cooling the stop with liquid nitrogen. In addition, both the stop and the specimen are kept at a low temperature before sawing is begun. We have found that the combination of these two measures prevents tissue from thawing while being sawed.

Equipment needed for this procedure includes a deep-freeze capable of reaching -70°C and a modified band saw. Construction is uncomplicated and the cost of the materials modest (approx. US $200.00). The device, therefore, can be rated simple, reliable and inexpensive.

SUMMARY AND CONCLUSIONS

Tissue slices of 2-3 mm uniform thickness are needed for the E12 technique. When large specimens are sawed to these dimensions, friction generated by the saw blade tends to soften the tissue and render the sections unacceptable. This paper describes a means of modifying a commercially available band saw so that the tissue being sectioned is cooled with liquid nitrogen. Construction is relatively easy and inexpensive (US $200.00). We have found that using this instrument according to the procedure recommended results in completely acceptable sections.

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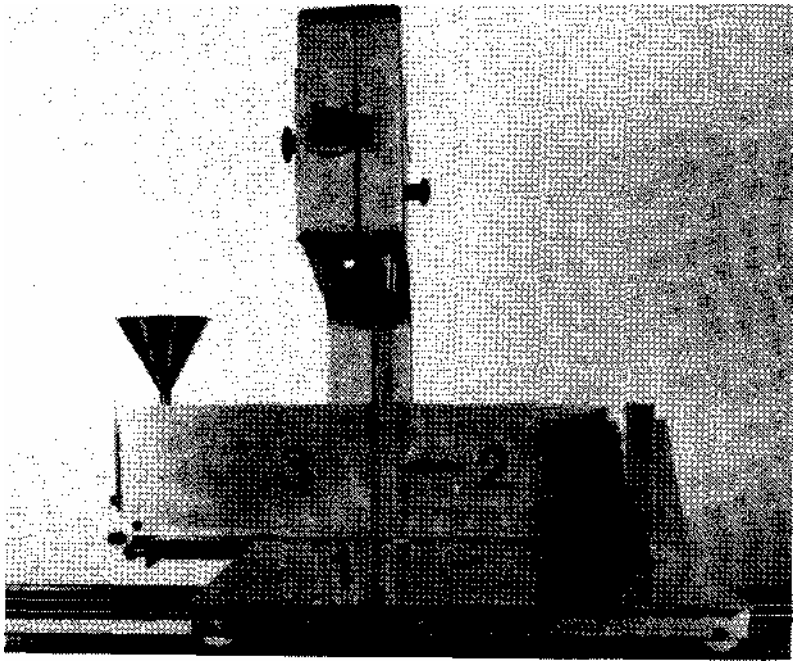

Fig. 1 A view of the complete saw. 1 table, 2 blade, 3 stop.

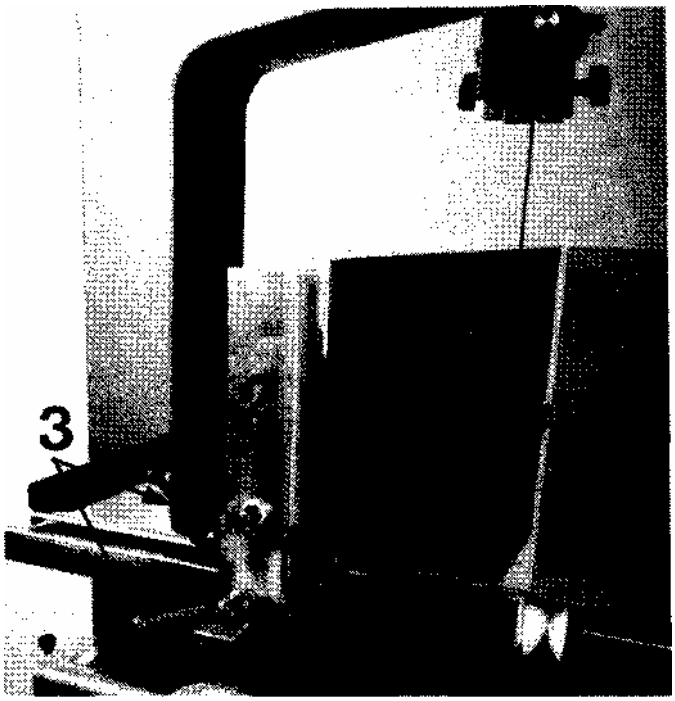

Figure 2.

The stop, as seen from above and to one side. 1 stop, 2 receptacle for liquid nitrogen, 3 setscrews.

Figure 3.

Side elevation of the stop. 1 blade, 2 stop, 3 receptacle for liquid nitrogen.