

Fig. 2 *The new pump harnessed to the old.*

drive sprocket, through the guide bearing on the new box, and had a second female coupling secured to its free end with allen screws (Figure 2).

To provide additional rigidity, and to maintain the drive shaft location, the complete unit, consisting of the original pump and new platen unit, was mounted on a formica-covered wooden base.

The motor in the original pump, which is four years old, has proved adequate to cope with the additional load.

Voltage Stabilizer Transformer Unit

To operate two colorimeters simultaneously, the original 25-watt output Volstat was replaced by one with a 50 watt output, but with the same input and output voltage. This unit was purchased from Advance Components Ltd. for £10 10s 0d, and was located and wired up in the same position as the original.

Other Modules

A second set of dialyzer plates and accessories, colorimeter, and recorder, purchased from Technicon Instruments Co., was set up according to the manufacturer's instructions.

I wish to thank Mr Ryan, hospital group engineer, and his staff, and Mr Greer for their engineering work, and Dr Wade, consultant pathologist, for encouraging this project.

An embedding and sectioning technique for immunohistochemical studies of minute specimens of tissue

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Freezing very small tissue blocks, eg. needle biopsies, and cutting these specimens, are fairly difficult techniques. Besides, attention must be paid to storage of these frozen tissue specimens as repeated study may be necessary. In order to obtain and to keep frozen tissue in a good morphological state the following are necessary precautions: (1) Fixation and refixation on microtome chuck for cryostat sectioning must be possible. (2) Thawing, even only partially, and damaging of the tissue must be absolutely impossible. (3) Interference with histochemical reactions must be excluded.

Although several embedding techniques for minute tissue specimens which fulfil the above conditions are known, eg. in a gelatin-sephadex mixture (Bonomo, Tursi, and Del Zotti, 1964) we developed an embedding method which made the fixation on the cryostat microtome chuck, the cutting of morphologically good sections, and the storage of the tissue specimens quite easy.

Method

Before a minute tissue specimen is received, 10% gelatin (Difco) solution in saline is prepared and kept fluid at +25°C. The tissue specimen is placed in a gelatin capsule no. 3 (Lilly, Basingstoke, Great Britain). A little fluid gelatin is dropped on the bottom and against the side wall of a thin metal container and the gelatin capsule with the tissue specimen is placed on the cooling gelatin lump to keep it upright. Then the gelatin capsule is carefully filled with fluid gelatin (Figure 1). After this procedure the tissue is immediately snap-frozen, preferably in liquid nitrogen (-196°C), by sinking about half of the metal container into the freezing medium. Free inflow of liquid nitrogen into the metal container must be avoided, as the gelatin capsule may then crack.

For sectioning purposes a specimen holder from an ultramicrotome (Reichert) is fixed on a cryostat chuck (Figure 2). When the specimen holder is kept at -20°C in the cryostat, the frozen

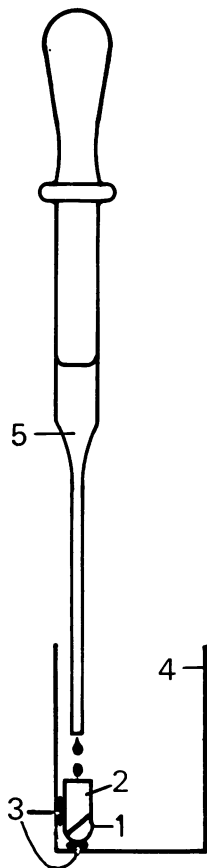


Fig. 1 Tissue specimen (1) in a gelatin capsule (2), which is kept upright by congealing gelatin (3) on the bottom, against the side wall of a small metal container (4). By filling the capsule with gelatin (5) the tissue is embedded.

gelatin capsule, which is easily removed from the bottom of the thin metal container, can at any time be clenched in the microtome specimen holder by simple screwing, without any chance of damaging or partially thawing the tissue (Figure 3).

Morphologically good sections are obtained by cutting through the capsule, gelatin, and tissue (Feltkamp-Vroom, Ruys, and Feltkamp, 1969). It may even be possible to cut ultrathin sections with an ultramicrotome equipped with a specimen holder cooled by liquid nitrogen. The

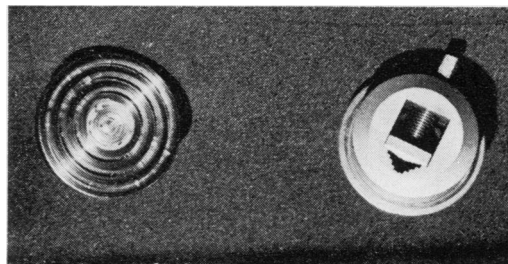


Fig. 2 A cryostat chuck (left) to which an ultramicrotome specimen holder (right) is fixed.

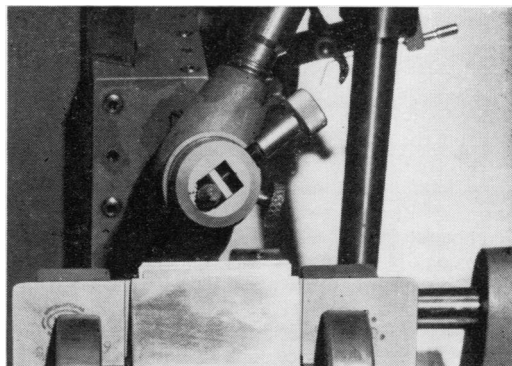


Fig. 3 For sectioning, the capsule with the tissue is fixed on the microtome by simple screwing.

tissue specimens are stored in their gelatin capsules and take little room.

References

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