Technical methods

Reconstitution of dried-up tissue specimens for histological examination

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Many histologists, however well organized their laboratories may be, must have had the unfortunate experience of discovering that an interesting or valuable specimen, set aside for future study, has dried up in its container. Such specimens usually appear rather grey or black, perhaps with an encrustation of salt if they have been stored in formol-saline, and rattle thinly if the container is shaken. Despite these unpromising appearances attempted salvage for histological examination may be worth while.

Experience gained during the study of dried and mummified tissues from ancient Egypt, pre-Columbian Peru, the Canary Islands, etc., has shown the importance of preliminary rehydration of the desiccated material (Sandison, 1955, 1957, 1963). It therefore seemed logical to apply similar techniques to the recovery of accidentally dried up laboratory specimens.

METHOD

The dried tissue should be placed in a generous volume of rehydrating fluid made up as follows:—

- 96% Ethyl alcohol .................................. 30 vol.
- 1% Aqueous formaldehyde ...................... 50 vol.
- 5% Aqueous sodium carbonate .............. 20 vol.

This fluid is slightly modified from that recommended by Ruffer (1911). A light brown colour may diffuse into the liquid from the specimen. The period of treatment is entirely empirical: the specimen must be examined from time to time and as soon as it becomes pliable it should be transferred to 10% aqueous formal saline for 24 hours. The time of treatment in the rehydrating fluid is usually measured in hours and rarely requires more than one day. If rehydration is inadequate, subsequent cutting of sections will prove difficult; if the specimen is left too long in the rehydrating fluid it may disintegrate.

The specimen should be processed by a double embedding method using a five-day histokine cycle (Russell, 1956). Cutting sections should not be difficult but a softening fluid such as Mollifex (B.D.H.) may be used if necessary. All conventional staining methods may be attempted; if routine haemalum and eosin is not satisfactory latent detail may be brought out by the phosphotungstic-acid-haematoxylin or Heidenhain's iron-haematoxylin methods.

Occasionally surgical biopsies are received from general practitioners in distant places in an unfixed and dried-up state and these may be treated in a similar manner. However, where drying is only superficial, it is simpler to treat

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Preparation of Gels

These are poured into trays consisting of a standard Perspex frame (0.25 in. thick with 8.25 in. × 5.0 in. outer dimension and 7.25 in. × 4.0 in. inner dimension with rounded corners) supported on a Perspex baseplate (0.25 in. thick and of 8.5 in. and 5.5 in. dimensions) clamped together with Bulldog clips.

APPARATUS FOR SLICING GELS

1 CUTTING APPARATUS Figure 1 gives the details and dimensions of the Perspex platform and pillars for mounting the dermatome blade (McIndoe pattern).

2 GEL SUPPORT These consist of rectangular pieces of sheet Perspex, \( \frac{1}{4} \) and \( \frac{1}{4} \) inch thick, shaped to fit firmly but easily into a Perspex frame. They serve the purpose of raising the gel above the surface of the cutting platform so that it lies proud to the cutting blade (Fig. 1, E). The thickness of the gel support selected allows the precise thickness of the gel slice to be pre-determined.

3 A shallow dish approximately 12 in. square and 3 in. deep, filled with water.

PROCEDURE

1 After removal from the electrophoretic tank, the left-hand corner of the anode end of the gel is perforated with a small cork borer. This allows accurate orientation of the gel slices during subsequent manoeuvres.

2 Separate the gel from the sides of the frame with a sharp knife.

3 Place a spare baseplate over the exposed surface of the gel and invert the tray so that the original baseplate can be removed. If there is any tendency for the gel to stick to this plate, removal is facilitated by submerging the tray and gel underwater in the shallow dish.

4 Lift the Perspex frame away and fit a gel support into its window so that it has a flush fit on one side. Replace this over the gel, re-invert, and place on the cutting platform. Slip the far end of the frame under the blade which is allowed to lie unclamped in its Perspex supports.

5 Place a second frame exactly over the first so that the cutting blade is sandwiched between them. Compression of the blade between the two frame ends ensures that the blade lies in a horizontal plane. Firmly secure the blade in the Perspex supports by tightening the thumb screws.

6 Exerting slight pressure from above slowly advance the two frames so that the knife slides smoothly between them.

7 Remove the top frame. The top slices can now be separated from the main body of the gel by immersing underwater in the shallow dish. Before staining the top slice requires to be inverted to expose the cut surface.

Gels of any thickness can be sliced using the above apparatus providing they are prepared in the standard Perspex frame, 0.25 inches thick. By using a starch block 0.5 in. in thickness, it is possible to obtain three to four slices from the same block. This allows a variety of studies involving different staining techniques to be made on the same protein samples.

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FIG. 1. Section of reconstituted specimen of synovium to show a blood vessel and inflammatory cells. Haemalum and eosin × 670.

by immersion in normal saline for an hour or so until the appearance returns to a more natural state. The specimen may then be transferred to fixative in the usual way (Lendrum, 1951).

RESULTS

The histological appearances of the dried-up specimen will approximate more or less closely to those commonly seen.

A piece of synovium from a patient with rheumatoid arthritis was mislaid for three years. When discovered it was black and quite unrecognizable. Nevertheless following rehydration for 24 hours the typical macroscopic frondose pattern of synovium was readily seen.

Sections stained routinely with haemalum and eosin showed in the fronds well-preserved blood vessels and focal inflammatory cells (Fig. 1). Higher-power examination revealed the characteristic appearances of plasma cells and even the chromatin pattern of the nucleus was seen to be maintained (Fig. 2). Clearly, if fixation has been adequate, even detailed structure may be well preserved after total gradual desiccation.

REFERENCES

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