

## Technical methods

### Localisation of sulphated glycosaminoglycans in the mucopolysaccharidoses by a simple technique using cryostat sections

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The sulphated glycosaminoglycans (GAG), which accumulate in the mucopolysaccharidoses, are difficult to localise because of their high solubility in water and alcohol water mixtures. A number of metachromatic methods using 'fixation' in organic reagents have been described.<sup>1,2</sup> In our hands, these have given poor localisation due to diffusion of GAG during staining. This would occur if the amount of water in the dye solvent, which is sufficient to allow reaction between GAG and dye, also allowed diffusion of GAG. The presence of dye at the site of GAG before the introduction of water should provide the optimum condition for the prevention of diffusion. On this basis the following technique has been developed.

Cut two 15-20  $\mu\text{m}$  cryostat sections, pick up on slides, and immediately place one into absolute ethanol saturated with toluidine blue (about 1%) for at least 10 minutes. Place the other into 10% formol saline for 10 minutes, rinse in tap water, and transfer through 70%, 90%, and absolute ethanol to absolute ethanol saturated with toluidine blue. Leave in this for 10 minutes. Stain both sections as follows:

- 1 1% toluidine blue in 50% ethanol in water—10 minutes;
- 2 dehydrate rapidly in absolute ethanol;
- 3 clear in xylene and mount in a resinous mounting medium.

### Results

Alcoholic toluidine blue 'fixed' sections of skin from a 21-week-old fetus affected by type I mucopolysaccharidosis showed cells containing metachromatic granules. The cells were of two types, cells typical of fibroblasts containing a few granules which could

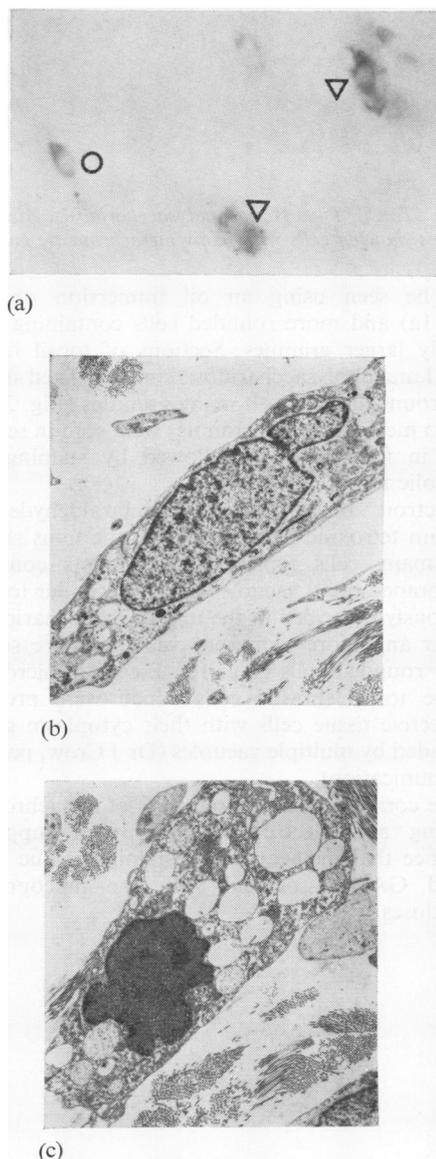
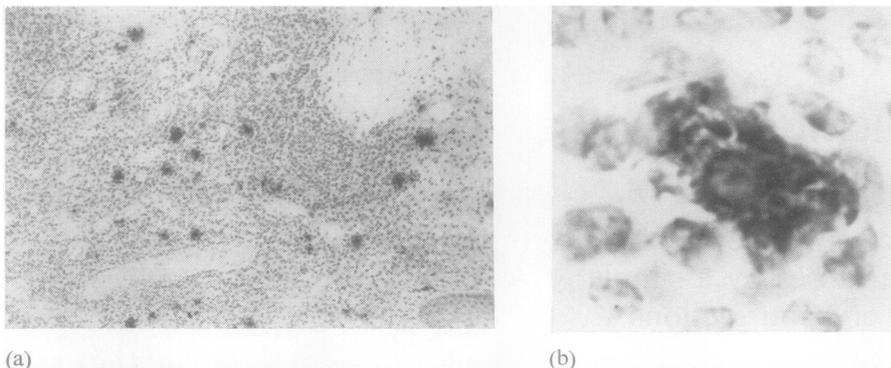


Fig. 1 *Fetal skin. Type I mucopolysaccharidosis: (a) alcoholic toluidine blue stained cryostat sections  $\times 1000$  showing a fibroblast-like cell with a few metachromatic granules (○) and more rounded cells with many granules (△). (b) and (c) electron micrographs  $\times 3000$  showing similar cells containing vacuoles.*



(a) (b)  
**Fig. 2** Tonsil. Type II mucopolysaccharidosis. Alcoholic toluidine blue stained cryostat sections showing cells with many metachromatic granules (a)  $\times 80$ ; (b)  $\times 1200$ .

only be seen using an oil immersion objective (Fig. 1a) and more rounded cells containing many slightly larger granules. Sections of tonsil from a type II mucopolysaccharidosis similarly fixed showed only rounded cells with many granules (Fig. 2a and b). No metachromatic granules were seen in sections fixed in formol saline followed by staining with alcoholic toluidine blue.

Electron microscopy of glutaraldehyde and osmium tetroxide fixed skin from the fetus showed that many cells typical of fibroblasts contained membrane-bound vacuoles (Fig. 1b) similar to those previously described in the mucopolysaccharidoses.<sup>3</sup> Larger and more numerous vacuoles were seen in a few rounded cells (Fig. 1c). Electron microscopy of the tonsil showed cells which were probably connective tissue cells with their cytoplasm greatly distended by multiple vacuoles (Dr J Crow, personal communication).

The correlation between results of metachromatic staining and electron microscopy is supportive evidence that metachromatic staining is due to the stored GAG associated with the mucopolysaccharidoses.

In both type I and type II mucopolysaccharidoses the stored substances are dermatan sulphate and heparan sulphate.<sup>4</sup> Other types store one or both of these with the exception of type IV, in which the GAG is keratan sulphate. It seems likely that this simple technique should be suitable for studies of all the mucopolysaccharidoses.

#### References

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- <sup>4</sup> Gantz M, Gehler J. The mucopolysaccharidoses. Inborn errors of glycosamino glycan catabolism. *Human Genetics* 1976;**32**:233-55.

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