Phosphate buffer at pH 8.0 gave the sharpest staining reactions, although there was little difference at pH 7.0 or pH 7.5. As the buffer pH was increased above pH 8.0 staining with both substrates became progressively weaker, especially above pH 9.0. Below pH 7.0 staining with α -naphthyl butyrate became weaker, and below pH 5.0 staining with naphthol AS-D chloroacetate began to disappear.

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Simple technique to identify haemosiderin in immunoperoxidase stained sections

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Identifying iron compounds in immunoperoxidase stained sections usually presents little or no problem to the trained eye. The distinction between peroxidase positive staining and haemosiderin becomes important when one is in doubt as to which compound is giving the "brown positive" result.

Recent biopsies have presented this problem. Although naturally occurring endogenous peroxidase activity has been blocked in the sections there has been confusion between the brown staining of haemosiderin and the positive brown staining of the 3-3' diaminobenzidine tetrahydrochloride, especially in weakly positive sections.

The staining of serial sections with haematoxylin and eosin and by Perls' Prussian blue is routinely performed when this problem arises. But unless a comparator microscope is used to check the identity of the stained cell, the problem remains. Counterstaining the peroxidase stained slide with Perls' Prussian blue technique, however, permits the

identification of the two compounds on the same slide.

Material and methods

Sections were cut from biopsy specimens of surgically removed pituitary gland which had been embedded in paraffin and fixed in formalin. Ten 3 μ m serial sections were prepared and dried in 37°C incubator for 12 h.

The immunoperoxidase staining method used was an adaptation of the method of Kovacs *et al.*¹ The method differed from the original technique by using antihuman prolactin antiserum (Mercia Brocades) at a dilution of 1/1000 (diluent 0.15 M phosphate buffered saline, pH 7.2) at 4°C for 1 h on material obtained from surgical biopsies. Before staining, the natural endogenous peroxidase activity was blocked using the modified technique that Slocombe *et al.*² used in the demonstration of blood group antigens.

After treatment with 1% osmium, the last stage of the original technique before mounting the section, the sections were gently washed in running tap water for 5 min. They were then washed several times in distilled water. The original technique of Perls³—heated potassium ferrocyanide and 1% hydrochloric acid—was then applied to show the haemosiderin in the section.

The Perls' Prussian blue and the uncounterstained peroxidase serial slides were compared with the peroxidase section counterstained with Perls' Prussian blue using a Zeiss comparison bridge mounted on two Zeiss Standard 18 microscopes. (Carl Zeiss (Oberkochen) Limited)

Results

The three slides of the serial sectioned material—peroxidase uncounterstained, Perls' Prussian blue, and the peroxidase counterstained with Perls' Prussian blue—were carefully compared by matching the architecture of the fields using a comparison bridge. Neither the prolactin nor the haemosiderin components seemed to be diminished as a result of the extra staining procedure.

Low background staining resulted from the use of reasonably fresh potassium ferrocyanide crystals; the background staining appeared to increase in proportion to the increased shelf life of the crystals, although this was not measured histoquantitatively. But distinction could still be made between the peroxidase positive staining and the haemosiderin.

The increased time in washing after the osmium treatment, when performed with care, did not seem to increase the fragility of the sections, although more vigorous washing for longer periods was not attempted.

Discussion

Hormone identification plays an important part in the diagnosis of pituitary adenomas. Often, biopsy specimens obtained from surgically removed pituitary gland contain sites of old haemorrhage which are sparse, diffuse, and intermingled with the hormone producing cells. At the sites the differentiation between hormone and haemosiderin must be clear, which is why the technique was originally evaluated.

It has previously been used successfully after the peroxidase-antiperoxidase method of Sternberger and Cucullis⁴ in renal cell carcinoma,⁵ where large amounts of iron pigment were present in the section.

With the increased use of immunoperoxidase techniques in surgical pathology,⁶ the addition of this simple but old technique to the modern one could further increase its specificity.

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Storage of skin biopsies at -70°C for future fibroblast culture

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It is difficult to know when to establish fibroblastic cell cultures from paediatric necropsies. Newborn babies who seem to have defects due to chromosome aneuploidy may die before the results of lymphocytic karyotypes are known. Lysosomal storage diseases requiring confirmation by enzyme assay on cultured cells may be suspected only when microscopical examination of tissues has been completed. Establishing a culture from every necropsy is

expensive. A simple method of storing a tissue sample for subsequent culture would be useful in these circumstances and would also have other practical uses in a clinical cytogenetics or tissue culture laboratory—for example, when samples are delivered at inconvenient times.

The cryoprotective effect of glycerol on frozen spermatozoa was reported in 1949.¹ This resulted in many techniques which describe the storage of frozen mammalian cells and tumours.²⁻⁴ In 1959, dimethyl sulphoxide was used to prevent the haemolysis of frozen red blood cells.⁵ Five years later Lehr *et al*⁶ described the successful transplantation of skin autografts which had been previously frozen using cryoprotective agents.

We report here a simple method of storing skin biopsies ($2-3\text{ mm}^3$) at -70°C in culture medium plus dimethyl sulphoxide for 15-23 days without loss of capacity for fibroblastic cell growth.