Demonstration of intracytoplasmic needle-like inclusions in hepatocytes of patients with porphyria cutanea tarda

KR James, JM Cortés,* and FJ Paradinas
Charing Cross Hospital and Medical School, London; *Clinica de la Concepción, Universidad Autónoma, Madrid, Spain

Porphyria cutanea tarda (PCT) is a bullous dermatosis associated with liver damage and accumulation of uroporphyrin and heptacarboxyloporphyrins in the liver. This accumulation leads to red autofluorescence of unfixed air-dried sections with ultraviolet light and to the presence of needle-like inclusions in hepatocytes which are thought to represent crystallised porphyrins and which appear specific for, and therefore diagnostic of, PCT.

There are many reports of the light microscopy of the liver in PCT, and it is surprising that whereas in some of them inclusions were seen in almost every patient, no mention of them is made in most series. It appeared to us significant that inclusions have been reported to disappear during most staining procedures other than a rapid haematoxylin and eosin, and we felt that this could be due to their being dissolved by one of the reagents used.

Accordingly, a paraffin block of 10% formalin fixed liver from a case of PCT was examined for the presence of such inclusions. Sections were cut at 5 μm, floated briefly on warm water, picked up on slides, and dried at 37°C overnight.

One section was dewaxed in xylene and mounted in Ralmount. Numerous brown, needle-shaped birefringent crystals were clearly visible when examined microscopically. When examined with the fluorescence microscope using BG12 and BG38 exciter filters and a K530 barrier filter, strong red fluorescence broadly confined to the same areas as the crystals was seen. A further section was dewaxed, placed in absolute ethanol for 1 hour, cleared in xylene, and mounted. The results were similar to those of the first section.

A series of sections were next dewaxed and taken down to running tap water. They were left washing for 2, 5, 10, 20, 40, and 60 minutes, then dehydrated, cleared, and mounted. The crystals were totally removed after 10 minutes. The red fluorescence

†RA Lamb, 6, Sunbeam Road, London NW10 6JL, UK.

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Acicular (needle-like) intracytoplasmic inclusions viewed (a) with and (b) without polarised light. Modified Cole's haematoxylin (see text) × 1000.
Technical methods

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References


Requests for reprints to: Mr KR James, Department of Histopathology, Charing Cross Hospital Medical School, Fulham Palace Road, Hammersmith, London W6 8RF, UK.

A punch for Guthrie papers

WJ Revill and RH Wilkinson Department of Chemical Pathology, John Radcliffe Hospital, Oxford OX3 9DU, UK

Although there are numerous punches for removing small discs from Guthrie papers1* we needed a punch to remove the whole of the square section containing the blood spot (Fig. 3c). On each Guthrie card we require four circles to be filled with the blood; a maximum of two are required for the amino acid screen technique2 for the detection of excess phenylalanine in phenylketonuria. We added thyroid stimulating hormone (TSH) estimation to the screen for the diagnosis of hypothyroidism in the newborn. The case number of the patient is written on each of the last two squares. They are punched from the Guthrie card and sent to the regional assay laboratory for TSH estimation. This punch speeds up the preparation of the squares.

The machine

The punch consists of a frame of aluminium alloy (Fig. 1). Above the frame is a perforated table with a backstop. The table is raised above the base plate

*See HM (69) 72 para 7 request form/filter paper (form HMR 101/6).

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