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# Demonstration of needle-shaped hepatic inclusions in porphyria cutanea tarda using the ferric ferricyanide reduction test

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**Summary.** In 18 of 19 biopsy and 2 of 3 autopsy samples of hepatic tissue from cases of porphyria cutanea tarda needle-shaped cytoplasmic inclusions are capable of reducing ferric ions in the ferric ferricyanide reduction test. Thanks to the resulting Turnbull's Blue the inclusions are clearly visible, which facilitates their histological demonstration. In 20 biopsy and 20 autopsy samples of hepatic tissue from cases other than porphyria cutanea tarda the inclusions are not present. These needle-shaped inclusions are thus considered to be a specific histological feature. The ferric ferricyanide reduction test represents a simple method for their visualization, which can be used in routine diagnostic practice.

**Key words:** Porphyria – Liver – Cytoplasmic inclusions – Reduction test

## Introduction

Needle-shaped cytoplasmic inclusions in hepatic cells are seen as a specific histological feature in porphyria cutanea tarda (PCT) (James et al. 1980; Cortéz et al. 1980; Chlumská et al. 1981; Kemmer et al. 1983). In most cases of PCT, staining with haematoxylin and eosin does not reveal the inclusions because of their solubility in water (James et al. 1980). To enhance their demonstrability it is necessary to reduce the contact with water but even with this modified procedure the inclusions are visible only at high magnification and thus escape attention.

We have proved that these needle-shaped inclusions in PCT are capable of reducing ferric ions in the ferric ferricyanide reduction test. The reaction is considered to depend on the reduction of ferric to ferrous ions in the presence of ferricyanide, with the production of Turnbull's Blue (Lillie and Donaldson 1974). In hepatic tissue from PCT cases, the resulting blue colour visualizes all needle-shaped inclusions, even those not visible in haematoxylin-eosin staining. We decide to investigate whether the demonstration of the inclusions with the reduction test could be used for histological diagnosis of PCT in hepatic tissue.

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## Material and methods

We examined 19 needle biopsies of hepatic tissue from patients suffering from PCT and hepatic tissue samples from 3 deceased PCT patients. The controls were 20 liver biopsies from patients with chronic active hepatitis and alcoholic liver disease and liver samples from 20 autopsies without PCT, 10 of them with cirrhosis.

After routine formalin fixation, five-micron thick paraffin sections were performed. The ferric ferricyanide reduction test was performed as originally designed by Golodetz and Unna (1909) or Schmorl (1928) or in Lillie's modification (Lillie 1965). The staining method recommended was that paraffin sections were deparaffinized in xylene and brought through graded alcohols to water. They were then placed in freshly prepared ferric ferricyanide reagent: 30 ml 1% ferric chloride solution, 10 ml fresh 1% potassium ferricyanide solution, for 5 min. After washing in running water for 2 minutes they were counterstained in 1% Nuclear Fast Red solution for 1–5 min. After rinsing in distilled water they were dehydrated quickly in 96% and 100% alcohol, cleared in xylene and mounted. Needle-shaped inclusions appeared in blue and the colour remained stable for several weeks.

In addition, the sections were stained with Nuclear Fast Red and by the modified haematoxylin-eosin method with a short contact with water during staining (James et al. 1980). The Perls' reaction was used for demonstration of ferric ions and Lillie's technique with potassium ferricyanide for demonstration of ferrous ions (Lillie 1965). The sections were examined in polarized light and their primary fluorescence established.

To prove that the reducing inclusions are identical with those visible in oversight staining, the sections from one biopsy containing a large number of inclusions were stained by the

modified haematoxylin-eosin method and ten microscopic fields were recorded at high magnification. The reduction test was then performed, the same fields were examined and again recorded. To establish the water-solubility of the inclusions the deparaffinized sections were washed for one hour under running water and then either the reduction test or staining with Nuclear Fast Red only were performed.

## Results

After the ferric ferricyanide reduction test needle-shaped cytoplasmic inclusions capable of reducing ferric ions occur in a majority of PCT liver tissue samples. No inclusions were found in control samples without PCT (Table 1). In the hepatocellular carcinoma, present in one of the autopsy specimens, no inclusions are found, although they are present in the surrounding hepatic tissue. The inclusions are thin needles of unequal length, bright blue after the reduction test. They are found in the cytoplasm of hepatocytes mostly in clusters, less frequently isolated (Fig. 1). In some instances their length is equal to the diameter of the hepatocyte. The clusters sometimes almost completely fill the cytoplasm. In the steatotic cells, the inclusions surround the fat vacuole. They were found several times in Councilman bodies. The number of inclusions in individual hepatic tissue specimens tends to vary: from isolated ones seen only at high magnification to large quantities visible even at low magnification.

The inclusions are not regularly distributed in hepatic tissue, but they are found in relatively circumscribed areas of varying size, which alternate with large areas without any inclusion. This phenomenon is particularly obvious in autopsy samples. The areas with inclusions occur often at the sites of fatty change and they are mostly adjacent to or overlapping with haemosiderin deposits. In biopsy samples no regular topographical relationship between the areas with inclusions and the hepatic lobule could be established. The cirrhosis present in our two positive autopsy samples makes topographical orientation somewhat difficult.

Sections from samples with reducing inclusions were stained by a modified haematoxylin-eosin method with a short contact with water and also with Nuclear Fast Red. In all these biopsy and autopsy samples of hepatic tissus we found, after a long search and at high magnification, yellowish brown inclusions, repeatedly described in PCT (Waldo and Tobias 1973; Grossman et al. 1979; James et al. 1980; Cortéz et al. 1980; Chlumská et al. 1981; Kemmer et al. 1983). Their yellow-brown colour is best visible after staining with Nuclear Fast Red. The cellular and tissue location

**Table 1.** Occurence of ferric ions-reducing needle-shaped inclusions in hepatic tissue

| | Number of cases | Inclusions | |
|---|---|---|---|
| | | Present | Absent |
| PCT biopsy | 19 | 18 | 1 |
| Control biopsy | 20 | 0 | 20 |
| PCT autopsy | 3 | 2 | 1 |
| Control autopsy | 20 | 0 | 20 |

PCT = Porphyria cutanea tarda

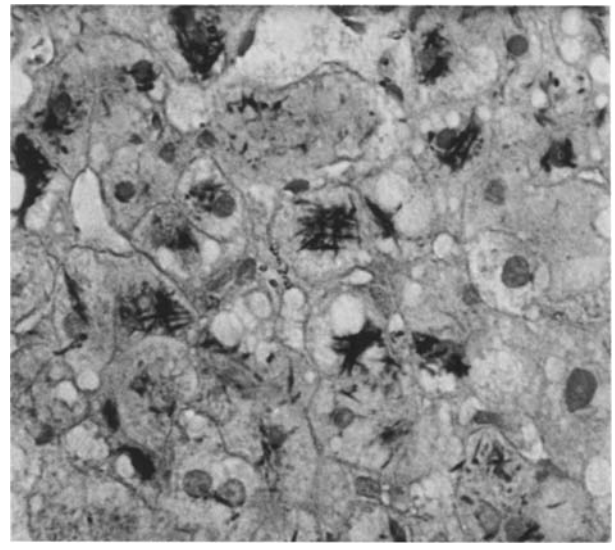

**Fig. 1.** Needle-shaped inclusions in cytoplasm of hepatocytes in porphyria cutanea tarda. Ferric ferricyanide reduction reaction, counterstained with Nuclear Fast Red, × 360

and the shape of these inclusions are identical with those of ferric ion reducing inclusions. In one biopsy we recorded the inclusions photographically after modified haematoxylin-eosin staining, in ten high-power microscopic fields. After having then performed the ferric ferricyanide reduction test we found ferric ion reducing inclusions in the same fields. The shape and location of these inclusions are identical (Fig. 2). All yellow-brown inclusions visible after haematoxylin-eosin staining react positively in the reduction test, while many reducing inclusions cannot be seen after haematoxylin-eosin staining only.

Washing under running water for one hour revealed that the solubility of inclusions from different specimens tends to vary. In 13 biopsies and one autopsy sample no inclusions could be found after washing, not even after the reduction test. In 6 biopsies and one autopsy sample the inclusions are visible after washing, even if in Nuclear

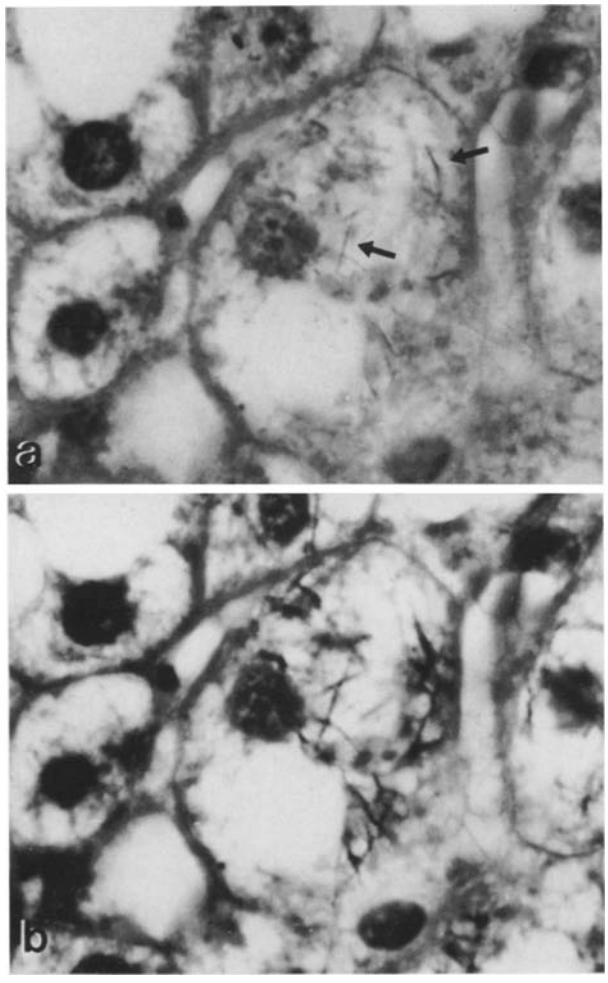

**Fig. 2a, b.** A liver cell containing a large number of inclusions. **a** Haematoxylin-eosin staining with a short contact with water: cytoplasmic inclusions as thin poorly-visible needles (*arrows*). **b** The same field after subsequent ferric ferricyanide reduction reaction: the inclusions are more readily visible, their shape and location are the same as in **a**. ×1200

Fast Red staining only. Their number is, however, reduced and their shape deformed.

Needle-shaped inclusions are non-reactive in reactions for demonstration of ferric and ferrous iron. Haemosiderin deposits, always present in PCT, are stained a dark blue colour by Perls' reaction for ferric ions and a pale bluish-green by Lillie's ferricyanide reaction for ferrous ions. In polarized light inclusions in sections stained with Nuclear Fast Red or in sections counterstained with Nuclear Fast Red after Perls' or Lillie's reaction present a weak birefringence. This birefringence is even weaker in sections stained by a modified haematoxylin-eosin method as well as in sections after the reduction test. The primary orange-red fluorescence, characteristic of porphyrins (Enerbäck and Lundwall 1970; Grossman et al. 1979; James et al. 1980), could not be demonstrated in paraffin-embedded tissue. The staining properties of inclusions are summarized in Table 2.

**Table 2.** Staining properties of needle-shaped inclusions in paraffin sections

| Method | Inclusions |
|---|---|
| Haematoxylin-eosin | mostly absent |
| Haematoxylin-eosin modified (reduced contact with water) | yellow-brown |
| Nuclear Fast Red | yellow-brown |
| Perls' reaction for ferric iron | negative |
| Lillie's reaction for ferrous iron | negative |
| Ferric ferricyanide reduction reaction | bright blue |
| Polarized light after Nuclear Fast Red or reactions for iron | weakly birefringent |
| Polarized light after H&E modified or reduction reaction | very weakly birefringent |
| Primary fluorescence | negative |

## Discussion

The needle-shaped inclusions reducing ferric ions have the same shape and cellular and tissue location as the yellow-brown inclusions described in sections from liver of PCT cases stained with haematoxylin-eosin (Waldo and Tobias 1973; Grossman et al. 1979; James et al. 1980; Cortéz et al. 1980; Chlumská et al. 1981; Kemmer et al. 1983). An analysis of identical microscopic fields after haematoxylin-eosin staining and after a subsequent ferric ferricyanide reduction test confirms that the inclusions visible in sections processed by these two methods are identical, i.e. they are the same cytoplasmic structures, yellow-brown in colour, which are able to reduce ferric ions. After the reduction test the inclusions become blue and much better visible. As a result it is possible to identify more needles and to define better their cellular and tissue location. We are unable to explain the irregular distribution of the inclusions in hepatic tissue, which is highly conspicuous. Similarly as Waldo and Tobias (1973) we could establish no topographic relationship between tissue areas rich in inclusions and the hepatic lobule.

The results confirm the findings obtained from haematoxylin-eosin stained sections (e.g. Cortéz et al. 1980; Kemmer et al. 1983) that needle-shaped inclusions are a specific feature of the liver in PCT. We have found none in biopsy and autopsy liver samples without PCT. According to the literature they are not present in other porphyria

types (Cortéz et al. 1980). Their absence in one biopsy and one autopsy samples with confirmed PCT indicates that a negative result does not exclude the diagnosis PCT.

The chemical nature of the inclusions is not known. It is usually assumed that they are crystalline porphyrins (Waldo and Tobias 1973; James et al. 1980; Chlumská et al. 1981). The reducing property of the inclusions is hardly ever mentioned in literature – some inclusions reduce silver in the Fontana-Masson method (Cortéz et al. 1980). The weak birefringence of the inclusions, observed after staining with Nuclear Fast Red, is almost invisible after both haematoxylin-eosin staining and the reduction test. In erythropoietic protoporphyria the birefringence of pigment deposits is due to protoporphyrins (Klatskin and Bloomer 1974). In contrast to PCT the distinct birefringence is also present after haematoxylin-eosin staining. It would thus appear that the optically active component of the inclusions in PCT is a different chemical compound.

In this study we did not consider the pathogenesis of the needle-shaped inclusions whose nature has not as yet been explained. Neither light microscopy of paraffin sections nor electron microscopy (Waldo and Tobias 1973; Chlumská et al. 1981; Kemmer et al. 1983) can inform us whether inclusions exist in hepatic tissue in vivo or whether they are an artifact, most probably of aldehyde fixation. Whether they are an intra vitam existing structure or a diagnostic artifact, they are a specific morphological feature of PCT, and the ferric ferricyanide reduction test is a simple method of demonstrating them by light microscopy.

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